METHANE OXIDATION IN TEMPERATE AND TROPICAL SOILS

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Declaration

This thesis has not been submitted in any other application for a degree and is the result of my own work and composition

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Abstract

Rates of methane (CH_4) oxidation (-) and emission were measured from a range of soil types and land uses to investigate the soil and environmental variables which affected the land-atmosphere exchange of CH_4. The influence of soil characteristics, land use, inputs of nitrogen (N) and sulphur (S), and temporal variations in soil water content and temperature on CH_4 flux were examined in the field using a static chamber technique, and in the laboratory using soil cores. The flux of CH_4 was measured from a range of sites in the UK, Cameroon and Borneo, including temperate and tropical forest, agricultural land, moorland and blanket bog.

In mineral soils, rates of CH_4 oxidation ranged from a maximum uptake of -27.2 ng m^{-2} s^{-1} in an undisturbed forest in Cameroon, to a small net emission of 0.8 ng m^{-2} s^{-1} from an agricultural field in Scotland. Rates of CH_4 oxidation, both in temperate and tropical climates, were found to be inversely correlated with soil bulk density, indicating that the rate of gaseous diffusion of CH_4 to methanotrophs was the primary factor controlling rates of CH_4 oxidation. Soil bulk density may therefore be used to predict rates of CH_4 oxidation: CH_4 oxidation rate (ng m^{-2} s^{-1}) = 25.1 × bulk density (g cm^{-3}) - 33.6 (r^2 = 0.786, $p<0.001$). Seasonal variability in CH_4 flux was observed and was determined by both soil water content and temperature. The influence of each variable was related to the soil type. Land use strongly affected CH_4 flux with rates of CH_4 oxidation from disturbed sites (logged forest or agricultural land) between 10 and 90 % smaller relative to undisturbed sites. Compaction (and the associated affects on the soil structure) and fertilisation (and resultant changes on N turnover) appeared to be the main factors responsible for the inhibition. Inputs of N between 40 and 260 kg N ha y^{-1}, through pollutant deposition and fertilisation respectively, were observed to inhibit CH_4 oxidation rates by up to 65%. Laboratory experiments showed that rates of CH_4 oxidation were inhibited by up to 87% following application of various N compounds (40 kg N ha y^{-1}), however, it was observed that the application of an equivalent amount of NaCl resulted in a similar inhibition.

In peats, soil water content controlled the magnitude and direction of flux within sites by affecting the degree of anaerobicity of the peat and hence the depth of the CH_4 oxidising layer. Other factors such as peat depth and substrate quality influenced inter-site variability. Water content determined seasonal variability in CH_4 flux. However, soil temperature was also shown to have a strong effect on CH_4 emission rates from peat monoliths when the water table was at a constant height (Ea's between 50 and 75 kJ mol^{-1}). Vascular transport was found to be important in the transport of CH_4 from zones of CH_4 production to the atmosphere. The largest CH_4 emission rate at 780.4 ng m^{-2} s^{-1} was observed through the vascular plant *Menyanthes trifoliata* in pool areas of the blanket bogs in Caithness. S application (100 kg S ha^{-1} y^{-1}) to peat monoliths, which were net emitters of CH_4, inhibited emission rates by up to 50% as a result of competitive inhibition between methanogens and sulphate-reducing microorganisms.

The contribution of CH_4 produced by termites to the CH_4 budget was investigated in undisturbed and disturbed forests in Cameroon and Borneo. In Cameroon a positive correlation was observed between termite biomass in the soil and the flux of CH_4 from or to the forest floor, with the direction of flux changing from a net sink to a source for CH_4 at a termite biomass greater than between 3.8 and 18.0 g m^{-2}, depending on the time of year and level of disturbance. Large rates of CH_4 emission up to 2000 ng s^{-1} $mound^{-1}$ were observed from individual termite mounds. CH_4 budgets were calculated based on surveys of the mounds and CH_4 flux measurements from the forest floor and from the mounds. All forest sites were net sinks for CH_4 at between -3.05 and -6.15 kg ha^{-1} y^{-1}. However, termite CH_4 emissions and deforestation significantly mediated the soil sink strength by up to 28 and 35% respectively.

In summary, rates of CH_4 oxidation from mineral soils were low and covered a small range relative to CH_4 emission rates from peat. Rates of CH_4 oxidation were significantly inhibited by anthropogenic disturbance such as deforestation, conversion to agriculture, and inputs of N. Spatial variability was controlled by the bulk density of the soil, both in temperate and tropical climates, demonstrating the importance of the gaseous diffusion status of the soil and the secondary role of temperature in regulating oxidation rates. Seasonal variability affected both CH_4 emission and oxidation rates. The relative influence of soil water content and temperature on the CH_4 flux varied between sites and was dependent on the soil type.

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Contents

Chapter One

Introduction

1.1 General overview

Methane (CH_4) is the most abundant hydrocarbon in the atmosphere and plays an important role in its radiative and chemical balance. The continuing increase in atmospheric CH_4 concentrations since the industrial revolution, concurrent with other greenhouse gases such as carbon dioxide and nitrous oxide, has led to speculation that the enhanced greenhouse effect resulting from these gases will cause a change in climate. Atmospheric CH_4 concentrations also effect the concentrations of other important trace atmospheric constituents including the hydroxyl radical, ozone and carbon monoxide. The atmospheric mass balance of CH_4 is determined by the balance between sources and sinks. The observed rise in CH_4 concentrations may be a result of increasing sources and/or decreasing sinks (Cicerone & Oremland, 1988, Khalil & Rasmussen, 1990, Prather, 1994). The largest single source of CH_4 is emission from natural wetlands, estimated at 115 Tg y^{-1} (IPCC, 1994). However, the majority of the source strength is anthropogenic in origin (Table 1.1). Methane is produced during the final stage of organic matter decomposition under anaerobic conditions by a group of bacteria known as methanogens. The largest sink for CH_4 is reaction with the hydroxyl radical OH in the troposphere. A small proportion of CH_4 is also transported to the stratosphere where it plays an important role in stratospheric chemistry as a source of water. The only terrestrial sink for atmospheric CH_4 is in soils where it is oxidised by microorganisms known as methanotrophs. This investigation was directed primarily to a study of the terrestrial sink for atmospheric CH_4, which has previously received little detailed attention.

Soils are a small but important sink for atmospheric CH_4. The rate of increase in atmospheric CH_4 is of a similar magnitude to the soil sink, currently estimated at 40 Tg y^{-1} (IPCC, 1995), and therefore any changes that occur in the soil sink strength will influence the rate of change of atmospheric CH_4. Rates of CH_4 oxidation have been measured from a range of environments including temperate and tropical forest soils, tundra, savannah, and agricultural land (Seiler *et al.*, 1984, Whalen & Reeburgh, 1990, Crill, 1991, Keller & Reiners, 1994, Flessa *et al.*, 1995). Relative to emission fluxes, rates of CH_4 oxidation cover

a small range and appear to be principally governed by soil texture, soil water content, nitrogen inputs and land use (Dorr *et al.*, 1993, Castro *et al.*, 1995, Sitaula *et al.* 1995, Dobbie *et al.*, 1996). Other factors such as temperature, organic matter content, pH and site fertility however, can also significantly affect CH_4 oxidation rates (Hŋtsch *et al.*, 1994, Czeipel *et al.*, 1995, Castro *et al.*, 1995). Methane oxidising microorganisms are also responsible for modifying the flux of CH_4 from zones of CH_4 production, such as wetlands, landfill sites, and termites to the atmosphere. Reeburgh *et al.*, (1993) estimated that soil processes account for 80% of global CH_4 oxidation when gross oxidation is taken into account.

1.2 CH_4 and the greenhouse effect

The earth's climate is dependent on the presence of naturally occurring greenhouse gases in the atmosphere, such as H_2O vapour, CO_2 and CH_4, to re-emit outgoing IR radiation back to earth (see Dickenson & Cicerone, 1986). Without the IR absorbing gases, Schneider (1989) calculated that the earth's average surface temperature would be about -18 °C. Anthropogenic increases in these and other gases such as N_2O, O_3 and CFC's has led to an enhanced greenhouse effect (IPCC, 1995). The atmospheric concentrations of the greenhouse gases CO_2, CH_4 and N_2O have grown by about 30%, 145% and 15% respectively since pre-industrial times. Mean global surface temperatures have increased by between 0.3 °C and 0.6 °C since the late 19th century, and sea levels have risen by between 10 and 25 cm over the last 100 years (IPCC, 1995). IPCC (1995) concluded that the observed climate change was unlikely to be entirely natural in origin.

Methane has a strong IR absorption at 7.66 :m, in a spectral region where CO_2 and H_2O absorb only weakly, so it has a direct effect on the radiative balance of the troposphere and stratosphere (Donner & Ramanathan, 1980, Dickenson & Cicerone, 1986). Kiehl & Dickinson (1987) calculated that on a per-moleculer basis CH_4 was approximately 25-30 times more effective as a greenhouse gas than CO_2. The effect of the presence of 1.5 ppm CH_4 in the atmosphere has been calculated by Donner & Ramanathan (1980) to result in the globally averaged surface temperature to be about 1.3 K higher than it would be with zero CH_4. Methane can also affect the radiative properties of the atmosphere through indirect means, such as tropospheric O_3 production (Isaksen & Hov, 1987), and the atmospheric oxidation of CH_4 to CO, which is eventually oxidised to CO_2. Cicerone & Oremland (1988) calculated that CO_2 from this source was about 6% of the CO_2 release from anthropogenic sources.

1.3 Atmospheric chemistry

Methane takes part in many chemical reactions in the troposphere and stratosphere, affecting the concentrations of other greenhouse gases and in particular the production and destruction of O_3. The main sink for CH_4 is its oxidation by tropospheric OH to CO and eventually to CO_2. However, the products of the reaction sequence depend on the atmospheric concentrations of NO_x. In a low NO_x environment the destruction of CH_4 may be accompanied by the destruction of O_3, whereas in a polluted environment with high NO_x concentrations (> 20 ppt) O_3 is produced. Crutzen and Graedel (1986) calculated that in a high NO_x environment there is a net production of 3.7 O_3 molecules and 0.5 OH radicals for each CH_4 molecule oxidised, whereas a net loss of 1.7 O_3 and 3.5 OH molecules may occur in a low NO_x environment.

A proportion of CH_4, estimated at between 32 and 48 Tg y^{-1} (IPCC, 1994), is transported to the stratosphere where it plays an important role in stratospheric chemistry, reacting with O_3 destroying Cl atoms and forming HCl (Cicerone & Oremland, 1988). Methane transport to the stratosphere and its subsequent oxidation is also an important source of stratospheric water vapour, the most effective greenhouse gas. Blake & Rowland (1988) calculated that an increase in mean atmospheric CH_4 concentrations from 1.0 ppm to 1.684 ppm has caused an increase in stratospheric water vapour of 28%.

Because OH is the primary atmospheric sink for CH_4, increases in CH_4 concentrations can affect the concentration of OH in the atmosphere, therefore providing a feedback affecting the rate at which CH_4 is destroyed (Sze, 1977). Thus it has been suggested that the contemporary increase in atmospheric CH_4 has probably decreased OH concentrations (Thompson & Cicerone, 1986), while OH decreases may be responsible for part of the temporal increase in atmospheric CH_4 concentrations (Cicerone & Oremland, 1988).

1.4 Atmospheric CH_4 concentrations

Historical changes in atmospheric CH_4 concentration, deduced from analysis of air trapped in ice cores and global observation networks, have approximately doubled over the last 200 years (Figure 1.1), rising from about 700 ppb two to three hundred years ago (Craig & Chou, 1982, Rasmussen & Khalil, 1984, Stauffer *et al.*, 1985) to its present level of approximately 1720 ppb in 1994 (IPCC, 1995). This rise is strongly correlated with global human population (IPCC, 1992). Etheridge *et al.* (1992) observed that the CH_4 concentration has increased from 823 ppb in 1841 to 1480 ppb in 1978, with the exception of the period

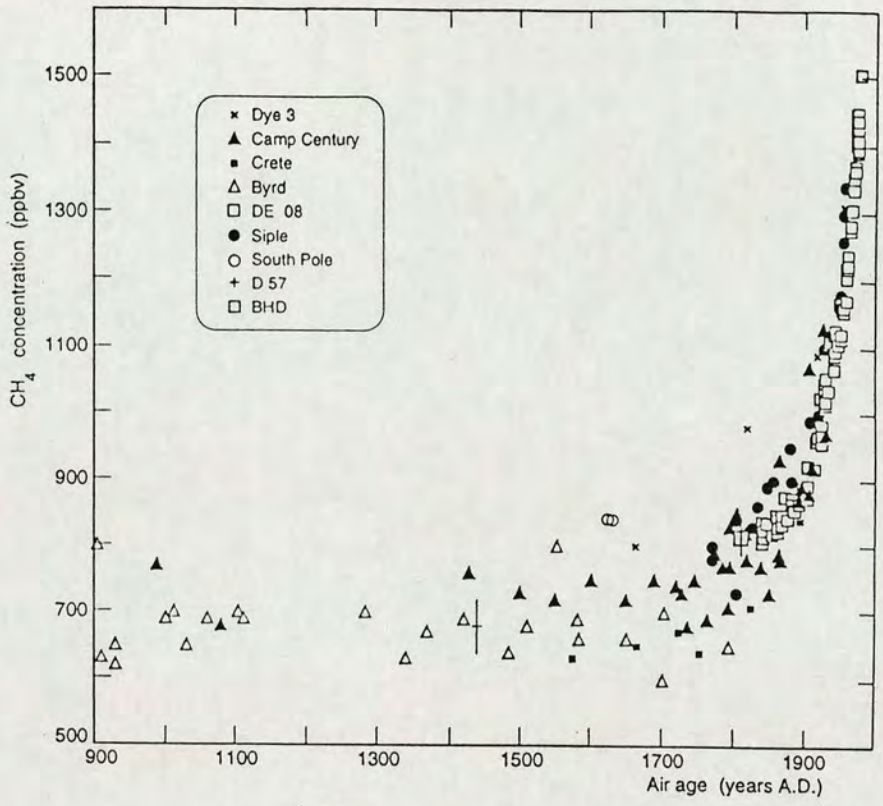

Figure 1.1. Atmospheric CH_4 concentrations over the last 1000 years from ice core data. Figure from Raynaud & Chapellaz (1993).

of the period between 1920 and 1945 when the growth rate stabilised to about 5 ppb y^{-1}, attributed to the effect on fossil fuel production of the First and Second World Wars, and the great depression. The source and sink strengths of CH_4 are discussed in 1.7 and 1.8. Atmospheric CH_4 concentrations were observed to be rising by approximately 16 ppb y^{-1} between 1978 and 1987 (Blake & Rowland, 1988). However measurements of atmospheric CH_4 concentrations over recent years have indicated a decline in the rate of CH_4 increase. Dlugochenky *et al.* (1994a) observed that the growth rate of CH_4 fell from 13.5 ppb y^{-1} in 1983 to 9.3 ppb y^{-1} in 1991 and observed that the growth rate in 1992 in the northern hemisphere was only 1.8 $\pm$ 1.6 ppb (Dlugochenky *et al.* 1994b). In 1994 the atmospheric CH_4 growth rate returned to approximately 0.8 ppb y^{-1} (IPCC, 1995). The reasons for this decline are not certain, but Steele *et al.* (1992) and Dlugochenky *et al.* (1994b) suggested that changes in fossil fuel exploitation could result in the observed rapid deceleration in rates of increase of atmospheric CH_4 concentrations.

The analysis of air bubbles trapped in ice from Greenland and Antarctica has provided a paleo-atmospheric record for several greenhouse gases, including CH_4, stretching back approximately 160 kyr BP (Jouzel *et al.*, 1987, Stauffer *et al.*, 1988, Chapellaz *et al.*, 1990) and in the case of Jouzel *et al.* (1993) 220 kyr BP. Atmospheric CH_4 concentrations have been observed to double between glacial (350 ppb) and interglacial (650 ppb) periods (Stauffer *et al.*, 1988) and have been observed to be in phase with glacial/interglacial conditions deduced from isotopic compositions of ice cores (Jouzel *et al.*, 1987, Chapellaz *et al.*, 1990). The relative contributions of orbital forcing, versus variations in atmospheric CH_4 (and other greenhouse gas) concentrations, on glacial/interglacial cycling is unknown. Chapellaz *et al.* (1990) observed a good relationship between the CH_4 periodicities and the orbital variations of the earth. The potential feedback effect of glacial to interglacial warming on atmospheric CH_4 concentrations has been discussed by Raynaud *et al.* (1988), who suggested that the impact of climatic change, increased temperature and extended deglaciated area on freshwater wetlands could have accounted for the large increase in atmospheric CH_4 concentrations during interglacial periods. However, Chapellaz *et al.* (1990) observed that the interglacial increase in atmospheric CH_4 occurred when much of the northern wetlands were ice covered and suggested that low latitude wetlands were responsible for the observed change.

1.5 Atmospheric distribution of CH_4

There is a distinct interhemispheric and seasonal cycle in atmospheric CH_4 concentrations, and the magnitude of the seasonal variability varies with latitude (Steele *et al.*, 1987). Atmospheric CH_4 concentrations are approximately 6% lower in the southern hemisphere than in the northern hemisphere, which corresponds to an excess northern hemisphere source of about 280 Tg y^{-1} (IPCC, 1994). These inter-hemispheric differences have been attributed to the fact that the major sources of CH_4 lie in the northern hemisphere (IPCC, 1994). The observed seasonal variations in atmospheric CH_4 concentrations are thought to be due primarily to the annual variation in tropospheric OH concentration and, to a lesser extent by the seasonal variability of some sources and atmospheric transport (Steele *et al.*, 1987, Dlugochenky *et al.*, 1994a).

1.6 The global methane budget

The observed increases in atmospheric CH_4 concentrations show that sources exceed sinks by about 35 to 40 Tg every year (IPCC, 1995). The sources and sinks are shown in Table 1.1 and are discussed below.

1.6.1 CH_4 sources

Methane is formed by the decomposition of organic matter under strictly anaerobic conditions by a group of bacteria known as methanogens, in environments such as peat bogs, rumens of animals and paddy fields. The processes and microorganisms involved are reviewed in Cicerone & Oremland (1988) and Oremland (1988). Abiotic processes such as biomass burning are also responsible for some CH_4 production. Anthropogenic emissions account for between 60 to 80% of the total global current emissions, and carbon isotope measurements indicate that about 20% of the total annual CH_4 emissions are related to fossil fuel production and use (IPCC, 1995). CH_4 is emitted from a wide variety of sources, discussed below. However, despite numerous investigations, many of the source strength terms are poorly understood and their estimates cover quite a wide range.

Natural Wetlands

The largest single global source for CH_4 is emission from wetlands, currently estimated to be between 55 and 150 Tg y^{-1} (IPCC, 1994). The emission of CH_4 from peat wetlands is discussed in Section 1.8.

Rice Paddies

The estimated contribution of rice paddies to the global CH_4 budget has been revised downwards in recent years, from 110 Tg y^{-1} (IPCC, 1990) to 60 Tg y^{-1} (IPCC, 1995). The publication of several studies covering a wider geographical area has led to an improved understanding of processes and better methods of extrapolation (Bachelet & Neue, 1993, Lal *et al.*, 1993, Shao *et al.*, 1994). Methane emission from rice paddies is influenced by soil temperature and water depth, agricultural practices such as fertilisation (Delwiche & Cicerone, 1993) and the growth stage of plants. Nouchi *et al.* (1994) estimated that 90% of the CH_4 produced in rice paddies is transported through the vascular system of plants to the atmosphere, thereby bypassing any oxidation which would have occurred had the CH_4 passed through the soil/water/atmosphere interface.

Enteric fermentation

Enteric fermentation by ruminant animals, both domestic and wild, represents a large source of CH_4, currently estimated at 85 Tg y^{-1}(IPCC, 1995) and is one of the better estimated

source terms in the CH_4 budget. Crutzen *et al.*, (1986) estimated that cattle contributed 74% of the CH_4 production by domestic animals and that CH_4 emissions from enteric fermentation had increased from 21 Tg y^{-1} in 1890 to 78 Tg y^{-1} in 1983. Emissions from domestic animals depend strongly on the amount and type of feedstuffs and manure handling systems employed (Lodman *et al.*, 1993).

Termites

Methane emission by termites has been subject to a wide range of estimates in recent years (Zimmerman *et al.*, 1982, Rasmussen and Khalil, 1983, Martius *et al.*, 1993). As a result of the scarcity of reliable field data, budgets have been calculated on the basis of rates of CH_4 emission from individual termites (Zimmerman *et al.*, 1982) or on CH_4 emission rates from only one feeding group (Rasmussen and Khalil, 1983, Martius *et al.*, 1993). The contribution of termites to the global CH_4 budget has been revised downwards from a maximum estimate of 150 Tg y^{-1} (Zimmerman *et al.*, 1982) to the present IPCC (1995) estimate of 20 Tg y^{-1}. However there are still many uncertainties associated with this source term, particularly with regards to extrapolation (Bignell *et al.*, 1996). Recent observations in West Africa have indicated that the biomass of the high CH_4 producing soil feeding species has been underestimated giving rise to the possibility that CH_4 emissions from the forest floor may represent a significant source of CH_4 (Eggleton & Bignell, 1995, Eggleton *et al.*, 1996).

Biomass burning

Biomass burning in tropical/subtropical areas is a major source of abiotic CH_4, currently estimated to contribute 40 Tg y^{-1} (IPCC, 1995) to the global CH_4 budget. The amount of CH_4 produced is dependent on the characteristics of the fire and the biomass material burned (Levine *et al.*, 1993). A review of the historical, economic and environmental aspects of biomass burning is given by Andreae (1993).

Landfills

Methane is produced during the anaerobic decay of waste matter dumped in landfills, currently estimated at 40 Tg y^{-1} (IPCC, 1995). The contribution of CH_4 emission from landfills is expected to increase due to population growth and urbanisation in developing countries (Schŋtz *et al.*, 1989). Production of CH_4 in landfills is strongly influenced by water content and also by refuse composition, microbial populations, available nutrients/toxins, temperature and pH (Bogner & Spokas, 1993).

Fossil fuel related sources

Fossil fuel CH_4 sources include coal and lignite mining, and pipeline transmission losses of

Table 1.1 The CH_4 budget (data from IPCC, 1994 and 1995).

	Individual Estimate ($Tg\ y^{-1}$)	Total ($Tg\ y^{-1}$)
(1) Identified sources		
(1a) Natural sources		
Wetlands	115 (55-150)	
Termites	20 (10-50)	
Oceans	10 (5-50)	
Other	15 (10-40)	
Total natural sources		**160 (110-210)**
(1b) Anthropogenic sources		
Fossil fuel related		
Natural gas	0 (25-50)	
Coal mines	30 (15-45)	
Petroleum industry	15 (5-30)	
Coal combustion	15 (1-30)	
Total		100 (70-120)
Biogenic carbon		
Enteric fermentation	85 (65-100)	
Rice Paddies	60 (20-100)	
Biomass burning	40 (20-80)	
Landfills	40 (20-70)	
Animal waste	25 (20-30)	
Domestic sewage	25 (15-80)	
Total		275 (200-350)
Total anthropogenic sources		**375 (300-450)**
Total identified sources		**535 (410-660)**
(2) Sinks		
tropospheric OH	490 (405-575)	
stratosphere	40 (32-48)	
soils	30 (15-45)	
Total sinks		**560 (460-660)**
Atmospheric increase		**37 (35-40)**
Implied sources (sinks + atmospheric increase)		**597 (495-700)**

natural gas, venting and flaring of gas wells and deposits and burning of petroleum products for fuel (Tyler, 1991). Kirchgessner *et al.*, (1993) estimated the global CH_4 emissions from coal mining to be 45.6 Tg in 1989 and Cicerone & Oremland (1988) estimated that 45 $Tg\ y^{-1}$ was released through gas drilling, venting and transmission, though they stated the present data was unsatisfactory. The low temperature combustion of coal has been suggested by Khalil *et al.* (1993) to be a significant source of CH_4. The IPCC (1995) currently estimate

CH_4 emissions from all fossil fuel related sources to be 100 Tg y^{-1}.

Oceans

The oceans at present are considered to be a small source of CH_4 of 10 Tg y^{-1} (IPCC, 1995). However, measurements are scarce, Cicerone & Oremland (1988) reviewed the subject and drew on the work of Ehhalt *et al.,* (1974 and references therein) who described the supersaturation of seawater with respect to the atmosphere, and an increase in CH_4 concentration with depth. Ehhalt (1974) also pointed out the possibility of CH_4 production from continental shelves and tidal flats. More recently Lambert & Schmidt (1993) reviewed the available measurements of CH_4 concentrations from near surface waters and also obtained an estimate of 10 Tg y^{-1}. They also calculated that the open ocean would become a sink for atmospheric CH_4 when the concentration of CH_4 in the atmosphere reached 2.25 ppm, due to the proportionality between the ocean source and the supersaturation of surface sea waters.

1.6.2 CH_4 sinks

The sinks for CH_4 are shown in Table 1.1 and are currently estimated to total 560 Tg y^{-1}, the vast majority of CH_4 being destroyed by the hydroxyl radical OH in the troposphere. Soils are the only terrestrial sink for CH_4 and as such play an important role in the budget.

Tropospheric OH

The global tropospheric concentration of OH radicals determines the oxidising capacity of the troposphere, the accumulation of radiatively trace gases and the flux of some O_3 depleting hydrocarbons to the stratosphere (IPCC, 1995). The primary source of the OH radical is the photolysis of O_3 by UV light at wavelengths less than 320 nm to produce excited oxygen atoms, followed by reaction between O(1D) and water vapour. Because of the very short lifetime of OH (1-2 s) (Lelieveld *et al.*, 1993), its concentration shows a high temporal and seasonal variability and therefore distributions and seasonal variations in OH concentrations are determined by photochemical models. Prinn *et al.* (1991) estimated that OH was present at 0.04 ppt in the troposphere. Most of the oxidation of long lived radiatively active gases such as CH_4 takes place in the tropics where high UV and humidity promote the formation of OH from photolysis of O_3 (Prather & Spirakovsky, 1990).

Stratosphere

A small proportion of CH_4, currently estimated to be 40 Tg y^{-1} (IPCC, 1995), is transported to the stratosphere where it plays an important part in stratospheric chemistry, being oxidised by OH and also reacting with Cl as described in Section 1.3.

Soils

Soils are a small but important sink for atmospheric CH_4, estimated to consume between 15 and 45 Tg y^{-1} (IPCC, 1995). Methane is oxidised under aerobic conditions by a group of bacteria known as methanotrophs. Methane oxidation is known to occur in a wide range of environments and can be inhibited by anthropogenic disturbance. Net fluxes and the processes which control oxidation rates are discussed below.

1.7 Soils as a sink for atmospheric CH_4

Methane oxidation rates have been measured from a range of environments including temperate forest soils (Steudler *et al.*, 1989, Crill, 1991), tropical forest soils (Keller & Reiners, 1994), savannah (Seiler *et al.*, 1984), tundra (Whalen & Reeburgh, 1990), and agricultural land (Flessa *et al.*, 1995, Dobbie *et al.*, 1996). Rates of CH_4 oxidation cover a relatively small range, relative to those of emission from wetlands, which makes extrapolation to the global scale more reliable. However, many uncertainties remain concerning the processes controlling CH_4 oxidation rates, in particular, the effect of N inputs, disturbance (in the form of deforestation and/or agriculture), and climate change. Also, the majority of measurements have been made in the Northern Hemisphere and many environments are poorly represented, in particular semi-arid and seasonally dry tropical ecosystems, estimated to consume the largest quantity of CH_4 annually (~38%) (Potter *et al.*, 1996). Budget calculations have been based on the dependence of CH_4 oxidation rates on soil diffusivity. Dorr *et al.* (1993), using soil textural class and a global data set of soil types, estimated the global soil sink strength to be between 9 and 56 Tg y^{-1}. The most recent budget for CH_4, using a simple model of diffusivity based on Ficks first law and a soil water balance model, suggested a global CH_4 consumption of between 17 and 23 Tg y^{-1} (Potter *et al.*, 1996) although this may be an underestimate due to the exclusion of frozen soils and some wetlands, both of which have been shown to be net sinks for at least part of the year (Harris *et al.*, 1982, Crill, 1991, Castro *et al.*, 1995).

Rates of CH_4 oxidation may be affected by a wide range of environmental variables, in general however, CH_4 oxidation rates cover a small range, typically from 0 to about -70.0 ng m^{-2} s^{-1}, (Dorr *et al.*, 1993, Crill, 1991) and the fluxes are significantly smaller, compared

to the flux from wetlands (which range from about -9.2 to 11200 ng m^{-2} s^{-1}, Bartlett *et al.*, 1993). However, as soil is the only terrestrial sink for CH_4, and because the rate of atmospheric increase is of a similar magnitude to the estimated soil sink strength, changes in the soil sink strength can significantly affect the CH_4 budget.

The principal reason that rates of CH_4 oxidation are relatively small and cover such a small range, and are not, for example, greater in the tropics than in temperate climates, is most likely to be a result of soil texture controlling the rate of diffusion of CH_4 to methanotrophs. Dorr *et al.* (1993) demonstrated the dominant control of soil texture on CH_4 oxidation rates over a range of soils in Europe, and others have also observed strong relationships with soil diffusivity (Keller & Reiners, 1994). However, several studies have demonstrated the importance of other environmental variables such as soil water content, soil temperature, soil organic matter content, pH and nitrogen inputs on CH_4 oxidation rates (Hηtsch *et al.*, 1994, Castro *et al.*, 1995, Czeipel *et al.* 1995). Several studies have investigated the effect of anthropogenic activities such as deforestation and conversion to agriculture on rates of CH_4 oxidation and have found consistent effects, with rates of oxidation significantly inhibited by these processes (Lessard *et al.*, 1994, Dobbie *et al.*, 1996, Goulding *et al.*, 1996). Seasonal variations in soil water content and temperature also affect rates of CH_4 oxidation (Crill, 1991), to an extent dependent on soil conditions, and may significantly affect annual oxidation rates.

A little investigated part of the CH_4 cycle is the oxidation of CH_4 from environments which are net emitters of CH_4, such as wetlands and rice paddies. Where oxic zones are present eg. in a wetland with a water table 10 cm below the surface, the CH_4 produced passes through the oxic zone where it may be oxidised by methanotrophs. Methanotrophs have been estimated to consume up to 80% of the CH_4 produced in wetlands (Oremland & Culbertson, 1992) and Reeburgh *et al.* (1993) estimated that CH_4 oxidation consumed 700 Tg y^{-1} when gross rates of oxidation were taken into account. The effect of climate change and anthropogenic effects on this process could have significant effects on the global CH_4 budget.

1.7.1 Microbiology of methanotrophy

The methanotrophs isolated so far, are a group of obligate aerobic eubacteria that grow only on CH_4 and/or one carbon compounds such as methanol. They are part of a larger group of microorganisms known as methylotrophs which are able to grow on a supply of reduced carbon compounds containing one or more carbon atoms but containing no carbon-carbon

double bonds (Anthony, 1986). The widespread occurrence of CH_4 oxidising bacteria in soil has been recognised since the 1920's (Hutton & Zobell, 1949), and the first pure culture of a methanotroph was isolated by S hngen in 1906. Whittenbury *et al.* (1970) isolated a wide range of methanotrophs from many different habitats which led to the first classification scheme.

Methanotrophs have been found in a wide and diverse range of freshwater, marine and terrestrial environments, wherever a source of CH_4 and O_2 is present (Whittenbury, 1970, Rudd *et al.*, 1975, Conrad, 1984, Ward *et al.*, 1987, Bedard & Knowles, 1989). As well as acting as a net sink for CH_4 in aerobic terrestrial environments, methanotrophs play an important role in mitigating the flux of CH_4 from aquatic habitats such as wetlands and rice paddies, and also from landfills (Reeburgh *et al.*, 1993). Anaerobic oxidation of CH_4 has also been measured from several environments (Alperin & Reeburgh, 1985, Reeburgh, 1983) and may be an important sink (Cicerone & Oremland, 1988). However, the process is not well understood and anaerobic CH_4 oxidising bacteria have yet to be isolated.

The methanotrophs oxidise CH_4 to methanol, catalysed by the enzyme methane mono-oxygenase (MMO), which requires molecular O_2 and a reductant NADH, followed by subsequent oxidation to formaldehyde, formate and ultimately to CO_2 (Anthony, 1986):

$$CH_4 \rightarrow CH_3OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2$$

Methanotrophic oxidation of CH_4 can be described as dissimilatory and assimilatory (Mancinelli, 1995). In dissimilatory pathways none of the carbon becomes cell biomass and during the complete oxidation of CH_4 to CO_2 only cellular energy is obtained. In assimilatory pathways CH_4 is oxidised and converted into cellular biomass via the assimilation of formaldehyde or formate into the cell.

The enzyme MMO which catalyses the initial stage of CH_4 oxidation to methanol has been subject to many investigations after the discovery that it was capable of inserting an oxygen atom into a wide variety of organic compounds including halogenated pollutants (Colby *et al.*, 1977, Wilson & Wilson, 1985). For a review of MMO see Lipscomb (1994). MMO is also responsible for the ability of methanotrophs to oxidise ammonia (O'Neill & Wilkinson, 1977, Bedard & Knowles, 1989) which has led to the hypothesis, from the observation that nitrogen inputs inhibit the CH_4 oxidising capacity of many soils, that NH_3 can compete with CH_4 for active sites on the MMO enzyme system (Bedard & Knowles, 1989). This hypothesis is discussed in more detail in Chapter 4.

Methane oxidising activity is also dependent on CH_4 concentration. The population of methanotrophs has been observed to increase with increasing CH_4 concentrations in a

landfill cover soil (Mancinelli, 1995). Bender & Conrad (1992, 1995) have examined the effect of CH_4 concentrations on the kinetics of CH_4 oxidation. They observed two CH_4 oxidising activities with different kinetic parameters in a soil which had been preincubated at high CH_4 concentrations: a high affinity activity with a low threshold concentration, adapted to low CH_4 concentrations, and a low affinity activity with a high threshold concentration. The bacteria responsible for the high affinity CH_4 oxidation, ie. those responsible for the oxidation of atmospheric CH_4, are unknown. For a detailed review of methanotrophy see Oremland (1988) and Anthony (1986).

1.8 CH_4 emission from peat wetlands

Numerous studies, mainly from the Northern latitudes, have provided a broad range of source estimates and an understanding of the processes controlling net CH_4 emission, namely water table height, temperature and substrate quality (Dise *et al.*, 1993, Roulet *et al.*, 1993, Nilsson & Bohlin, 1993). Many uncertainties remain, however, with CH_4 emissions from wetlands being characterised by very high spatial and temporal variability. Estimates of CH_4 emission from tropical wetlands are far less numerous, and Bartlett & Harriss (1993), reviewing the global CH_4 emission from wetland sources, calculated that CH_4 emission from tropical regions amounted to 66 Tg y^{-1} compared to 38 Tg y^{-1} from Northern wetlands.

The primary requirement for CH_4 production is anaerobiosis. Methanogens are strict anaerobes and require highly reducing conditions (E_h<-200 mV) for growth (Conrad, 1989). Methanogenesis is inhibited by O_2 through its toxic effects on methanogens. Water table height has been observed to be the primary controlling factor affecting rates of CH_4 emission in several studies (Dise *et al.*, 1993, Moore & Dalva, 1993). Methanogens are also strongly affected by temperature, with the majority having a temperature optimum between 30-40° C (Vogels *et al.*, 1988). CH_4 emissions from wetlands has been correlated with seasonal variations in temperature in many studies (Crill *et al.*, 1988, Dise, 1993). Most methanogens grow optimally over a relatively narrow pH range between about 6 and 8 (Oremland *et al.*, 1988). Despite the large quantities of CH_4 produced in acidic peatland environments (Bartlett & Harriss, 1993, Fowler *et al.*, 1996), no acidophilic methanogens have so far been isolated. However, Williams & Crawford (1985) isolated a methanogen that tolerated pH values as low as 3, but with an optimum between 6 and 7. Substrate quality and the botanical composition of the peat can also affect CH_4 production rates (Martikainen *et al.*, 1995, Nilsson & Bohlin, 1993). Organic matter inputs have been reported to increase

CH_4 emission in a paddy field (Schηtz, 1989), and organic matter from root exudates have also been associated with increased rates of CH_4 emission (Holzapfel-Pschorn & Seiler, 1986). The presence of alternate electron acceptors such as $SO4_2^+$ and NO_3^- have also been observed to affect the emission rates of CH_4 from wetlands (Westermann & Ahring, 1989, Fowler *et al.*, 1995). The transport pathway of CH_4 to the atmosphere can significantly affect net flux by affecting the degree of oxidation that occurs before reaching the atmosphere. Plant-dependent CH_4 transport has been estimated to contribute to up to 90% of the total flux (Whiting & Chanton, 1992, Yavitt & Knapp, 1995).

1.8.1 Microbiology of Methanogenesis

Methanogenic bacteria, along with the extremely halophilic and thermoacidophilic bacteria, comprise a distinct biological kingdom known as the Archaebacteria (Woese, 1978). Over 50 distinct species of CH_4 producing bacteria have been isolated from diverse anaerobic habitats, such as flooded soils, intestinal tracts of animals and freshwater and marine sediments (Jones, 1988), and also from more extreme environments such as seafloor hot vents (Anderson *et al.*, 1987) and hypersaline environments (Oremland & King, 1988).

Organic matter is decomposed under anaerobic conditions through a series of steps to form CO_2 and CH_4. Methanogens are only capable of metabolising a small range of substrates and are dependent on a consortia of microorganisms for their production. Conrad (1989) summarised the types of bacteria generally required for the anaerobic methanogenic degradation of organic matter as a) hydrolytic and fermenting bacteria, b) H^+ reducing bacteria, c) homoacetogenic bacteria and d) methanogenic bacteria. The breakdown of organic matter by these microbial groups provides the substrates (including hydrogen, carbon dioxide, acetate, formate, methanol and methylated amines) necessary for methanogenesis. Most methanogens are capable of using the H_2 mediated reduction of CO_2 to obtain energy, while the number of methanogens capable of growing on the other substrates is more restricted (Oremland, 1988). It has been estimated that in most organic rich methanogenic environments the major source of CH_4 is acetate and about one third of the total CH_4 formed is derived from the H_2 mediated reduction of CO_2 (Jones, 1988). However the energetics of the acetoclastic reaction are not well understood (Oremland, 1988). The utilisation of H_2 by methanogenic bacteria affects the metabolic activities of other bacteria involved in the decomposition of organic matter, a process known as "interspecies electron transfer", reviewed by Mah (1982). This process allows the partial pressure of H_2 to be kept at a suitable level for the fermentation of several organic

compounds. There is evidence that some bacteria live in symbiotic association with H_2 utilising methanogens, thereby allowing the fermentation of otherwise non-fermentable substrates (Conrad *et al.*, 1985).

The production of CH_4 is determined by the presence of electron acceptors such as O_2, NO_3^-, Fe^{3+} and SO_4^{2-} which are used up in a thermodynamically determined sequence and typically decrease with the distance from the oxic-anoxic interface (Conrad, 1989). Therefore the presence of alternative electron acceptors such as Fe^{3+}, SO_4^{2-} and NO_3^- can inhibit methane production by competing with methanogens for substrates (Westermann & Ahring, 1987). Particularly in marine environments, sulphate reduction has been observed to predominate over methanogenesis (Oremland *et al.*, 1988, and references therein) and evidence is accumulating that inputs of S and perhaps N from polluted atmospheres are inhibiting CH_4 emissions from peatlands (Fowler *et al.*, 1995, Nedwell & Watson 1995, Chapter 7). However, the presence of non-competitive substrates can result in both processes occurring simultaneously (Oremland, 1988). Detailed reviews of the biochemistry of methanogenesis may be found in Oremland (1988) and Vogels *et al.* (1988), and of the taxonomy and ecology in Garcia (1990).

1.9 Aims and layout of thesis

The principal aim of this investigation was to measure the net land-atmosphere exchange of CH_4, and to examine the parameters determining the magnitude of the flux on a temporal and spatial basis. The main focus was on CH_4 oxidation by aerobic soils where quantification of the CH_4 sink strength was required. Rates of CH_4 oxidation were measured from a range of soil types and land uses to investigate the soil and environmental variables which affected the land-atmosphere exchange of CH_4. The influence of soil characteristics, land use, inputs N and S and temporal variations in soil water content and temperature on CH_4 flux were examined in a range of sites in the UK, Cameroon and Borneo including temperate and tropical forest, agricultural land, moorland and blanket bog. From these measurements, and concomitant soil analysis, it was possible to establish some of the factors determining CH_4 oxidation rates. Long term measurements were also made, allowing the processes controlling the seasonal flux in the field to be identified. The work was part of a European consortium of research groups funded by the CEC's Environment Programme and specialising in complementary areas such as the microbiology of methanotrophs and the soil physical characteristics affecting CH_4 flux.

Investigations were also carried out into the factors which control CH_4 emission rates from peat bogs, as part of the NERC's Terrestrial Initiative in Global Environmental Research (TIGER) programme. Methane emission rates from peat wetlands are highly spatially and temporally variable and are likely to show large responses to anthropogenic influences such as acid deposition and climate change. Net fluxes were measured in the field and under controlled conditions in order to improve the understanding of the processes controlling CH_4 emission rates.

Another important part of the work, in collaboration with the TIGER consortium investigating the carbon balance in tropical forests, examined the balance between CH_4 produced by termites in mounds and soil, and CH_4 oxidised by forest soil. The work carried out in Cameroon by Eggleton *et al.*, (1996) suggested a large potential source of CH_4 from termites foraging within the soil profile. The balance between this CH_4 source and the oxidation capacity of the soil was investigated in disturbed and undisturbed tropical forest.

Following this Introduction, Chapter 2 presents the most common methods used throughout the thesis. More specific methods are discussed in the individual chapters. In Chapter 3 CH_4 flux measurements from a wide range of soils and land uses are described. The range in CH_4 flux observed was examined along with other environmental information such as soil analysis and land use history. This provided a basis for further investigation by identifying some of the major factors, such as soil bulk density, land use and nitrogen inputs, which affected CH_4 flux. Sites were then selected where these parameters could be investigated over long timescales in order to identify the parameters controlling CH_4 flux on a seasonal basis. These results are discussed in Chapters 4, 5 and 6. Chapter 7 deals specifically with CH_4 emission from wetlands. Chapters 8 and 9 report results from tropical soils and investigate the balance between CH_4 production by termites and its oxidation by soils. Measurements of CH_4 flux from tropical soils are particularly scarce, and this investigation provided a good opportunity to compare temperate and tropical fluxes. The location of the sampling sites is shown in Figure 1.2.

Chapters 3 to 9 are based on the form of scientific research papers. Five out of the seven chapters have been submitted to or accepted by refereed journals in a similar format to that which they are presented here. Details of the journals concerned are given under each chapter heading. Finally, in chapter 10 all the data are combined and the main processes controlling CH_4 flux on a temporal and spatial basis are discussed. The main conclusions are summarised in Chapter 11.

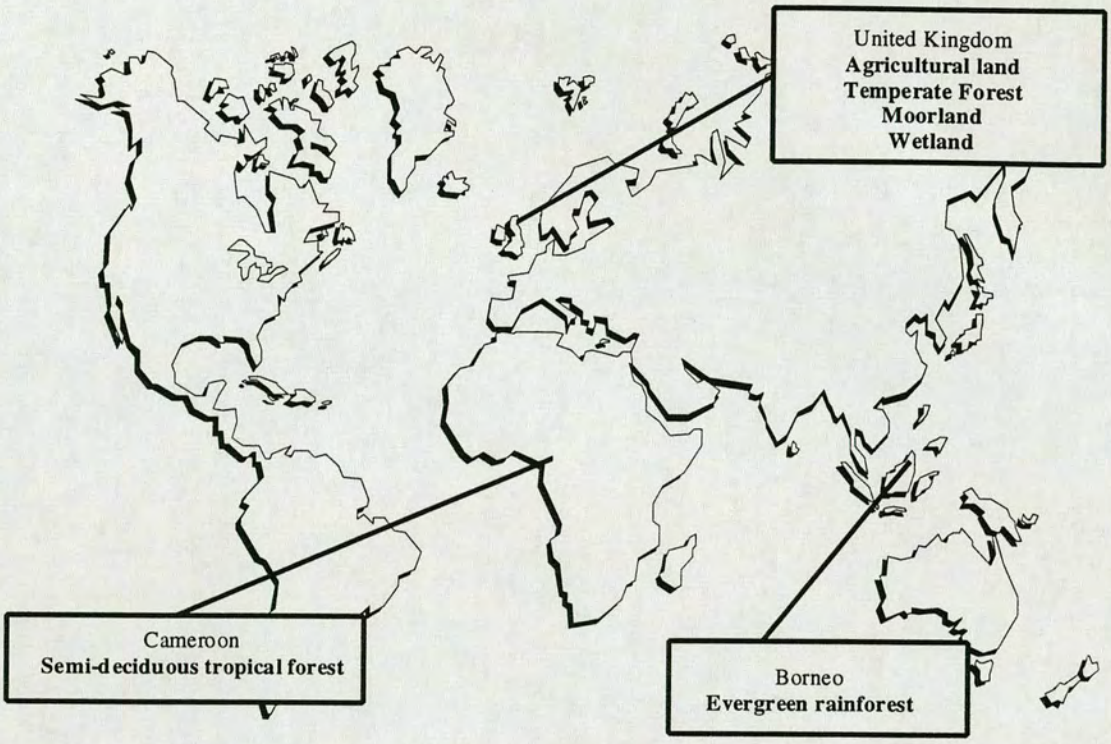

Figure 1.2. The locations of the sampling sites and the environments studied within each country.

Throughout the text negative numbers represent CH_4 oxidation rates and positive numbers represent CH_4 emission rates. The term 'flux' is applied to both CH_4 oxidation and emission.

Chapter Two

Materials and Methods

2.1 Introduction

The net land-atmosphere exchange of CH_4 is generally measured by either enclosure or micrometeorological techniques. Enclosure techniques use small chambers which cover an area of soil, allowing measurement of the change in concentration of the gas over a certain time period. Chamber techniques have several advantages, including their adaptability to many environments and variety of experimental requirements, low cost, and their sensitivity, allowing the determination of small fluxes which may be below the detection limit of other methods. They are very useful for process based investigations and were employed throughout this study where it was often necessary to measure CH_4 flux at regular intervals over long time periods in order to determine relationships between CH_4 flux and environmental variables. Micrometeorological methods integrate flux measurements over a much larger area, therefore smoothing the very large spatial variability often observed in chamber studies; however, the use of these techniques is often restricted by cost and fetch requirements. It was not possible to use micrometeorological methods for the measurement of CH_4 oxidation rates due to the low fluxes. A flux of greater than approximately 20 ng m^{-2} s^{-1} is required (K. Hargreaves pers. comm.). For reviews of micrometeorological methods see Fowler & Duyzer (1989) and Lenschow (1995).

Enclosure techniques using chambers have been used to measure trace gas fluxes from a wide variety of environments (Whalen & Reeburgh 1990, Keller *et al.,* 1990, MacDonald *et al.,* 1996) and for many different gases (Conrad *et al.,* 1988, Skiba *et al.,* 1994, Lessard *et al.,* 1994). Chamber design varies from the use of small bags to measure CH_4 emission from plants to the use of large tents for the measurement of CH_4 emission from termite mounds (Seiler *et al.,* 1984). Chambers are usually square or round in design, made from perspex, metal or plastic and cover between 500 to 900 cm^2 of soil (Livingston & Hutchinson, 1995). Chambers generally come under two categories, 'static' or 'dynamic', sometimes known as 'closed' or 'open' systems respectively. Static chamber measurements were used throughout this study and involve enclosing an area of soil and known volume of headspace air and relating the change in concentration of the gas to the area, volume and

time enclosed. The accuracy and precision of each measurement is dependent on the minimisation of a number of potential sources of error (see 2.2). Dynamic chambers involve passing a continuous flow of gas of known concentration over the surface of the soil until an equilibrium concentration gradient is established and measuring the inlet and outlet gas concentrations (Livingston & Hutchinson, 1995). The requirement for electricity and air pumps required to provide the flow-through conditions meant this technique was not suitable for many of the field sites used in this study.

In this study CH_4 fluxes were measured from a wide variety of environments ranging from the determination of CH_4 oxidation rates in temperate forest to the quantification of CH_4 emission from termite mounds. Site descriptions are given in each chapter. The static chamber technique was employed for all field studies (see 2.3.2) with some modifications for the measurement of CH_4 flux from peatlands (see 2.3.3). Laboratory studies to further investigate processes were also used and are discussed in relevant chapters. For example intact peat monoliths in controlled environment cabinets were investigated using a static chamber technique (Chapter 7), and a method was developed to measure CH_4 flux from small soil columns under controlled conditions, in order to investigate processes controlling CH_4 oxidation rates (Chapter 4). A simple atmospheric gradient technique was also employed to investigate whether areas of tropical rainforest acted as net sources or sinks for CH_4 (Chapter 10).

2.2 Sources of error associated with chamber measurements

2.2.1 Site Disturbance

One of the most potentially damaging influences of the method on accurate flux measurement is the installation of chambers into the soil. Great care must be taken when installing the chamber to avoid compaction which may alter the rate of gas exchange with the atmosphere and chambers should be installed with a cutting frame or knife to minimise disturbance. Root damage following insertion of chambers may affect nutrient availability to the soil microbial community (Matson *et al.*, 1990) and insertion of chambers into an environment where subsurface CH_4 production is occurring may facilitate CH_4 emission. Damage to plants involved in vascular transport may have a similar effect, therefore following chamber installation a suitable equilibration time should be allowed before measurements are taken. In this study CH_4 flux was measured 1 and 24 hours after chamber installation at several sites. Installation did not appear to influence CH_4 flux in aerobic soils but in some peats elevated ambient samples were observed. In general, and unless otherwise

stated in the following chapters, chambers were left to equilibrate for 24 hours prior to measurements being made.

2.2.2 Temperature effects

Induced temperature changes due to the chamber and the resultant modification of radiation may influence the microorganisms responsible for trace gas emission and uptake. The effect of chamber construction materials on enclosed air and soil temperatures has been demonstrated by Matthius *et al.*, (1980). However, the lag between air and soil temperature is such that effects can be minimised using short enclosure times, shading or insulating the chamber and applying a reflective covering to external surfaces (Hutchinson & Mosier, 1981). In this study, soil temperature was not found to be significantly different inside from outside the chamber over the enclosure times commonly used in this study (maximum 1 hour). On sunny days the chambers were shaded using an aluminium sheet. Similar results have been observed in other studies (Christensen *et al.*, 1996, U. Skiba pers. comm.), the lag time between air and soil temperature being sufficient to buffer any influence on the microbial community.

2.2.3 Pressure changes

A disadvantage associated with static chamber measurements is the exclusion of air pressure fluctuations. Gas transport across the surface-atmosphere boundary and within the sub-surface environment may be modelled as the sum of the mass flow and molecular diffusion (Livingston & Hutchinson, 1995). Chambers may be vented in order to transmit atmospheric pressure changes (Hutchinson & Mosier, 1981). Non-vented chambers were used throughout this study; however, the use of the flexible polythene lids (2.3.1) was intended to have a similar effect.

2.2.4 Concentration effects

The exchange of a trace gas across the soil - atmosphere boundary is largely a function of its diffusion coefficient and concentration gradient between sites of production or consumption and the soil surface (Hutchinson & Livingston, 1993). CH_4 oxidation rates typically follow first order kinetics (Whalen *et al.*, 1990) and non-linear models have been used to describe the rate of change of CH_4 concentration (Hutchinson & Mosier, 1981). However, Livingston & Hutchinson (1995) suggested that wherever possible measurement periods should be chosen such that the rate of concentration change can be assumed constant and therefore

modelled using linear regression. Matthius *et al.,* (1978) showed that chamber geometry strongly influenced soil gas exchange, demonstrating that chambers with small volume/basal area ratios exhibited more rapid concentration increases than enclosures with large ratios. In this study large chambers were employed and enclosure times were adjusted in order to compute fluxes using the following linear equation:

$$Flux_{CH4}\ (ng\ m^{-2}\ s^{-1}) = \frac{\Delta C \times 0.67 \times 10^{-3} \times v}{a \times t} \quad (1)$$

where Δ C is the change in gas concentration (ppb), 0.67 is a factor used to convert ppb to ng m^{-3}, v is the volume of the chamber plus lid (m^3), a is the area of soil enclosed (m^2) and t is the enclosure time (s). Linear models have been used in many studies (Steudler *et al.,* 1989, Whalen *et al.,* 1992, Ambus & Christensen, 1996) and were the preferred method of calculation in this study.

2.3 Field measurements using static chambers

2.3.1 Chamber design and materials

Chamber design was similar to that used by Clayton *et al.,* (1994) and consisted of a 20 cm length of polypropylene tube (Capper Plastics, Edinburgh, U.K.) with a diameter of 40 cm, fitted with a 4.5 cm wide outward facing polyvinylchloride (PVC) flange at one end and a bevelled edge at the soil insertion end to facilitate installation into the soil. The lid for enclosure consisted of a PVC flange fitted with a circular dome of polythene with a volume of 0.006 m^3 which minimised leakage when the gas samples were withdrawn. This lid allowed atmospheric pressure changes to occur without generating pressure differentials across the lid and permitted photosynthetically active radiation to pass through. A circle of hollow section draught excluder on the underside of the lid ensured gas tightness when the lid was secured to the chamber with metal spring clips (RS Components Ltd. Northants, UK.). The sample port consisted of a 20 cm length of 4 mm i.d. polythene tubing. A 3 way tap (MacKay & Lynn, Edinburgh, UK) was fitted to the tubing through which the chamber air was sampled. The chamber size was quite large relative to many studies of CH_4 oxidation rates (Livingston & Hutchinson, 1995) having a volume of 0.031 m^3 and covering an area of 0.126 m^2. Large chambers have the advantages of minimising surface disturbance and masking small scale spatial variability. A static chamber is shown schematically in Figure 2.1 and in the field in Plate 2.1.

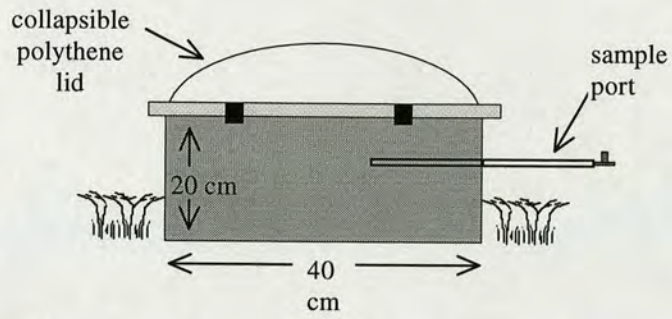

Figure 2.1 Schematic representation of a static chamber, typically having a volume of approximately 0.027 m^3 when inserted 3 cm into the soil.

2.3.2 Static chamber installation and measurements of CH_4 flux in the field

Chambers were installed using a stainless steel cutting frame and sharp knife which made a groove for the chamber to be inserted into while minimising the disturbance. The bevelled edge of the chamber was then placed over the groove and tapped into position to obtain gas tightness. Chambers were installed to a depth of between 2 and 5 cm and chamber heights were measured after installation to allow calculation of the internal volume of each chamber. Unless otherwise stated in the text, chambers were left to equilibrate for at least 24 hours before measurements were made in order to minimise the effect of the disturbance on gas exchange. Chambers were also shaded during sunny weather to minimise temperature effects.

Ambient gas samples were taken at time zero and chambers were enclosed for periods of up to one hour. The enclosure time was adjusted between sites depending on the rate of CH_4 oxidation or emission to ensure a linear concentration change. After the enclosure time the sample tube was flushed, and 500 ml of sample was withdrawn using a 1 l gas tight syringe (Hamilton, Phase Sep, Clywd,U.K.). Samples were collected into 1 l tedlar bags (KAD Detection Ltd, Glasgow, U.K.) and analysed for CH_4 within 24 hrs of collection using tunable diode laser (TDL) spectroscopy (see 2.4.1) during 1994 and gas chromatography (GC) fitted with a flame ionisation (FID) detector (see 2.4.2) throughout 1995. A comparison of the two methods of analysis is discussed in section 2.4.3. Samples of gas containing 2110 ppb CH_4 remained stable in the tedlar bags for periods of up to three weeks. The CH_4 flux was calculated using equation 1.

In order to ensure that the change in headspace concentration of CH_4 was linear with time during the measurement period for each site, replicate samples (n = 3) were withdrawn

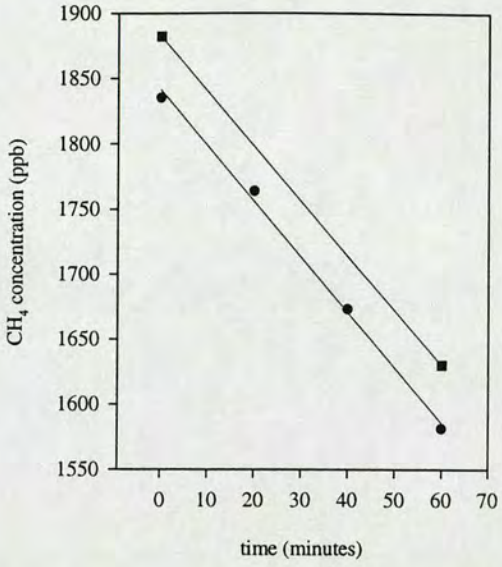

Figure 2.2 The concentration change of CH_4 in the headspace of a static chamber measured by GC (●) and TDL (■) at a grassland site, Glencorse.

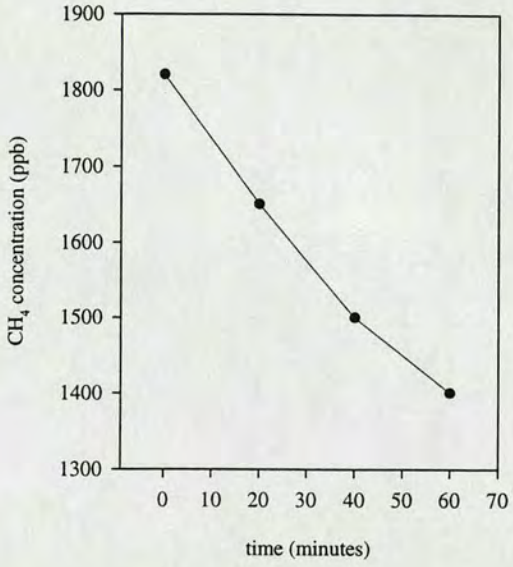

Figure 2.3 The concentration change of CH_4 in the headspace of a static chamber from a tropical forest soil in Cameroon

at 20, 40 and 60 minutes after enclosure into 5 ml gas tight syringes (Hamilton, Phase Sep, Clywd, U.K) and were analysed by GC. The results showed whether the change in CH_4 concentration (oxidation or emission) was linear over the measurement period (Figures 2.2 and 2.3). Figure 2.2 shows a comparison between analysis by GC and TDL. The decreases in headspace concentration can be seen to be linear over the measurement time ($r^2 = 0.998$). Both methods gave a very similar flux -10.9 and -10.7 ng m^{-2} s^{-1} for the GC and TDL respectively. The offset in the measurement of absolute CH_4 concentration by the TDL is discussed in section 2.4.3. In sites with a larger rate of CH_4 oxidation non linearity was observed, such as in the tropical forest soil measured in Cameroon (Figure 2.3) and in these circumstances the measurement period was shortened to cover the linear part of the concentration change only.

2.3.3 Measurements of CH_4 flux from wetlands

Measurements of CH_4 flux from wetlands are prone to disturbance from chamber installation and foot traffic. Therefore great care was taken in order to minimise disturbance. In boggy areas the cutting frame was not required and chambers were simply placed on the surface, the surface water providing an effective gas tight seal. Samples were taken as described above, and as remotely as possible with the use of long sample tubes. If the ambient samples contained elevated concentrations of CH_4 they were discarded on the

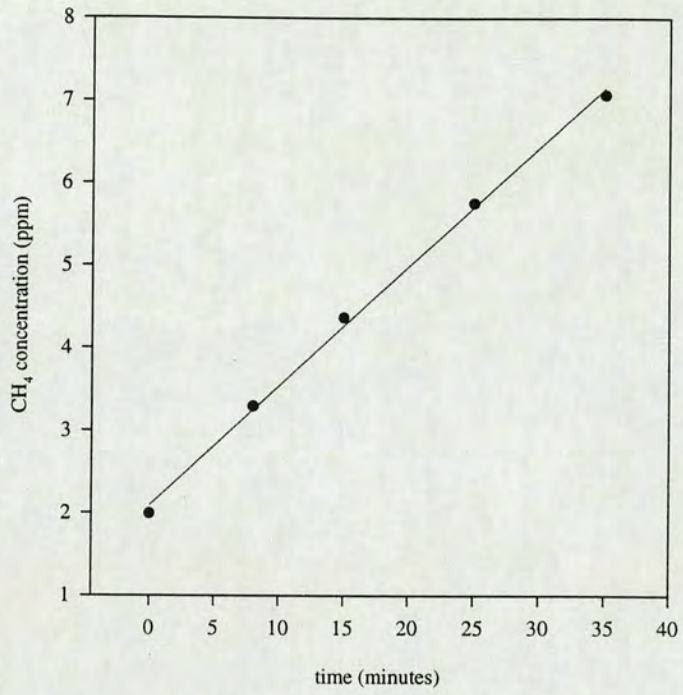

Figure 2.4 The change in CH_4 concentration in the headspace of a peat monolith maintained in a controlled environment room.

assumption that disturbance had induced ebullition. Sampling in the open pool areas was made possible by the use of a flotation collar made from water wings filled with Helium. This allowed the chamber to float while samples of gas were taken from, for example, vascular plants (Chapter 7). The enclosure time for measuring CH_4 emission was shortened, because of the large rates of exchange involved, typically to about 15 minutes. The linearity of CH_4 emission was investigated using the same method as for CH_4 oxidation described in 2.3.3 above. Figure 2.4 shows the emission of CH_4 from a peat monolith in a controlled environment room. CH_4 flux was linear (r^2 = 0.998) over the first 35 minutes with a concentration change of 5 ppm CH_4. An exponential equation fit to the points did not improve the goodness of fit.

2.4. Methods of analysis

2.4.1 Tunable Diode Laser Spectroscopy

Tunable diode laser spectroscopy has been subject to rapid development in recent years (Zahniser *et al.*, 1996) and in the study of trace gas fluxes has been most commonly applied to micrometeorological techniques. However, this type of instrument is also suitable for measuring discrete samples of gas. TDL spectroscopy is based on the differential absorption

achieved when an infrared laser is tuned on and off a spectral feature, usually a single vibrational/rotational line, of the target species (Kolb *et al.,* 1995). In this case the laser source was a lead salt tunable diode laser tuned to an absorption line at 3017.467 cm^{-1} which operated at a temperature of approximately 80 K and therefore required cryogenic cooling. The instrument consisted of a liquid nitrogen cooled dewar which housed the laser diode, an optical module for laser beam collection and transport, a multiple pass absorption cell and computer controlled electronics for laser frequency control and data acquisition (Zahniser *et al.,* 1995). The optical path is described in full by Zahniser *et al.,* (1995). Briefly, infrared light from the laser diode in the dewar is collected by a reflective microscope objective and focused on a pinhole, which defines the input aperture. The infrared beam is combined with a visible alignment beam from a helium-neon laser. The infrared light is then focused in a narrow beam into the optical absorption cell. The multipass absorption cell provides a long optical path length (36 m) in a compact volume thereby maximising the signal to noise ratio. The exit beam is reflected back to the dewar and is focused on an infrared detector. The data aquisition system stored concentrations directly to disc.

Samples were analysed using TDL spectroscopy (Aerodyne Research, Mas., USA) throughout 1994. The conditions under which the TDL was run for analysis of bag samples were as follows: pressure = 40 Torr, temperature = room temperature, flow rate = 0.5 - 1.0 l min^{-1}. The TDL is shown in Plate 2.1.

2.4.2 Gas chromatography

Chromatography is based on the partitioning characteristics of different compounds between a stationary phase (column packing) and a mobile phase (carrier gas) which flows through the stationary phase. A sample introduced into the mobile phase is carried through a column containing the stationary phase. Species in the sample undergo repeated partitions between the two phases until separation occurs. Separated components emerge in order of increasing interaction with the stationary phase. The least retarded component emerges first, the most strongly retained component elutes last. At constant temperature, pressure and carrier gas velocity the rate at which a component travels along the column is related to the partition coefficient K:

$$K = \frac{C_s}{C_g} \qquad (2)$$

where C_s and C_g are the concentrations in the stationary and mobile phases respectively. The separation of components is dependent on temperature, length and diameter of column,

packing material and the flow rate of the carrier gas. The components of a GC system are: i) the mobile phase or carrier gas which is pressure regulated and moves the sample throughout the system; ii) a sample injection system; iii) a separation column of appropriate length packed with an adsorbent material (the nature of which will depend on the species being analysed, common packing includes molecular sieve 5A, silica gel or porous polymers such as Poropak Q); iv) a detector of which there are a variety, Crill *et al.,* (1995), (a flame ionisation detector (FID) was used in this study. Detection of CH_4 is initiated when the compounds are pyrolysed in a H_2/air flame in the detector. The resulting ions and electrons set up a current which is measured across an electrode and an ion collector. Both column and detector were temperature controlled); v) a data recorder or integrator. For a full description of chromatographic methods see Smith & Arah (1991) and Crill *et al.,* (1995) and references therein.

Samples were analysed for CH_4 using GC with FID (Chrompack CP9000, the Netherlands) throughout 1995 and 1996. The column had a Poropack Q packing, a length of 2 m and an internal diameter of 4 mm and the carrier gas (N_2) operated at a flow rate of 40 ml min^{-1}. The column and detector operated at temperatures of 30 °C and 350 °C respectively. Peaks were quantified with a Spectra Physics integrator.

2.4.3 Accuracy and precision

For both methods of analysis a 2110 ppb compressed air working standard (Air Products, UK) was routinely used and was periodically calibrated against a 1990 ppb standard (Scott Speciality Gases) and a 3400 ppb standard (Air Products, UK). A 1000 ppb standard (Air Products, UK) from the Scottish Agricultural College was also analysed and showed a highly satisfactory intercomparison between laboratories.

The TDL had a precision of 0.05 %, equivalent to ± 1 ppb in ambient samples of approximately 1800 ppb. The GC had a precision of 0.7% equivalent to ∀15 ppb in 2110 ppb. The chamber measurement had a precision of 16 %, relative to a flux of 3.1 ± 0.5 ng m^{-2} s^{-1}. The precision was analysed by repeated measurements from the same chamber therefore disturbances in the soil CH_4 concentration profile, despite equilibration times between measurements, may have caused inaccuracies in the measurement.

Comparisons of the two methods for analysing CH_4 concentrations are shown in Figures 2.2 and 2.5. Figure 2.5 shows the concentrations of three standards analysed by GC and TDL. The concentration of CH_4 analysed by TDL can be seen to be offset by about 3% similar to the effect seen in Figure 2.2. The larger concentration observed by the TDL was

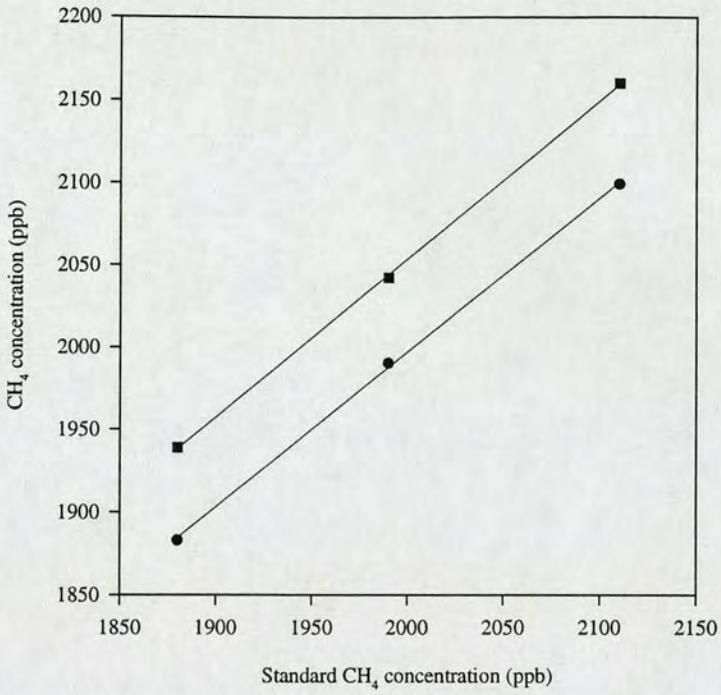

Figure 2.5 A comparison of standards between the GC (●) and the TDL (■).

caused by a problem with the pressure sensor and the anologue to digital convertor connecting the sensor to the personal computer which was used to compute fluxes. It was not possible to repair this fault, but because the calculation of fluxes depends on the calculation of concentration differences, the offset did not affect the calculation of fluxes.

2.5 Soil sampling and analysis

Three soil samples (top 10 cm) were taken and bulked, every time CH_4 fluxes were measured. Soil temperature at 5 cm depth was routinely measured during flux measurements using a hand held temperature probe (RS Components, UK). A subsample (2×10g) was analysed for water content (% dry weight) by drying in an oven for 24 hours at 105 °C. The soil sample was then frozen until analysis for soil available NH_4-N and NO_3-N. Duplicate samples of 15g soil were extracted (shaken for one hour) with 50ml 1M KCl, filtered using a Whatman No. 1 filter paper and then frozen (Crooke & Simpson 1971, Henrikson & Selmer-Olsen 1970). Analysis of the extract for NH_4-N and NO_3-N was carried out by the Environmental Chemistry Section at I.T.E., Merlewood using colorimetric analysis (Skalar 6000 photometer). Soil pH was measured in 0.01 M $CaCl_2$. Dry bulk density was calculated from the mass of oven dry soil in a given volume and organic matter was content was measured by loss on ignition.

Plate 2.1. Top - Static chamber, syringe and Tedlar bag as used in the field. Middle - Static chambers in the alder plot, Glencorse. Bottom - The TDL in the laboratory with the absorption cell in the foreground.

Chapter Three

Soil Environmental Variables Affecting the Flux of Methane from a Range of Forest, Moorland and Agricultural Soils

(A version of this chapter has been published in Biogeochemistry 34:113-132, 1996, with U. Skiba, L.J. Sheppard, K.A. Smith and D. Fowler as co-authors)

3.1 Abstract

Measurements of the net methane exchange over a range of forest, moorland, and agricultural soils in Scotland were made during the period April to June in both 1994 and 1995. Fluxes of CH_4 ranged from an oxidation of -12.3 to an emission of 6.8 ng m^{-2} s^{-1}. The balance between CH_4 oxidation and emission depended on the physical conditions of the soil, primarily soil water content. The largest CH_4 oxidation rates were found in the mineral forest soils, and CH_4 emission was observed in several peat soils. The smallest oxidation rate was observed in an agricultural soil. The relationship between CH_4 flux and soil water content found in peats was such that CH_4 oxidation occurred at soil water contents less than 325% (± 80%). CH_4 emission was found at soil water contents exceeding this value. A large range of CH_4 oxidation rates was observed over a small soil water content range in the mineral soils and CH_4 oxidation rates were negatively correlated with soil bulk density. Increased nitrogen loading of the soil due to N fixation, atmospheric deposition of N, and fertilisation, were consistently associated with decreases in the soil sink for CH_4, typically between 50 and 80%, on a range of soil types and land uses.

3.2 Introduction

Methane fluxes have been measured in a wide range of environments: temperate forests (Crill, 1991, Castro *et al.*, 1995), agricultural land (Mosier *et al.*, 1991, Flessa *et al.*, 1995), tundra (Whalen & Reeburgh, 1990), tropical forests (Keller *et al.*, 1990, Tathy *et al.*, 1993) and savannah (Delmas *et al.*, 1991). Rates of CH_4 uptake in soils appear to be influenced by a range of parameters including soil texture (Dorr *et al.*, 1993), soil climate (temperature, soil water content) (Whalen *et al.*, 1990, Castro *et al.*, 1994), land use (Lessard *et al.*, 1994, Dobbie *et al.*, 1996), N loading (Steudler *et al.*, 1989, Adamsen & King, 1993), balance of production versus consumption (Yavitt *et al.*, 1990) and soil conditions such as pH (Hŋtsch *et al.*, 1994). Investigations into the environmental variables influencing inter-site variability have mainly focused on the conversion of forest into agriculture which consistently shows a decrease in oxidation rates (Lessard *et al.*, 1994, Dobbie *et al.*, 1996). Castro *et al.* (1995) showed that CH_4 oxidation rates in forest soils were mainly determined by soil water content but, where soil water content was not limiting, site fertility controlled oxidation rates. Combinations of the processes controlling flux (ie. oxidation and/or emission) and their relative effects on different soils (e.g. peat vs. mineral soils), requires a greater understanding of the relative importance of the different environmental parameters and processes which control the exchange of CH_4 between soils and the atmosphere.

In this chapter measurements of CH_4 flux rates over a range of soils and environmental conditions during comparable time periods are reported. The results have been analysed to investigate the relative importance of a range of environmental variables on the net exchange of CH_4 in the field.

3.3 Materials and Methods

3.3.1. Site Description

Sites were selected on a variety of soil types and land uses throughout Scotland to provide a broad range of the environmental parameters affecting the magnitude and direction of CH_4 flux. The effect of enhanced N loading due to increased N deposition and N cycling was investigated by selecting sites with a range of N deposition along an altitude transect, and by including measurements from an N fixing alder plot. The effects of soil water content, texture, and land use were examined by measuring fluxes of CH_4 from agricultural, forest and moorland soils. Fluxes were measured over an area of blanket bog and forested blanket bog to quantify the scale of net CH_4 oxidation in a peatland environment. The measurement sites were as follows:

Glencorse (NT 241631) - This was an experimental field site near Edinburgh, S.E. Scotland (186m asl) which was converted from arable/pasture to forest in 1984. Sites were established in plantations of red alder, common birch, sitka spruce and rye-grass. Three chambers were installed randomly in each plot and CH_4 fluxes were measured on 20 and 30 June 1994. The soil was a brown forest soil of the Macmerry/Winton series (Brown & Shipley, 1982); the textural class was a clay loam.

Devilla (ND 065453) - Here the site was a lowland Scots Pine plantation near Kincardine, Fife, E. Scotland (65m asl), planted in 1952 on a brown forest soil, with gleying, of the Rowanhill association (Brown & Shipley, 1982). The soil was a sandy clay loam overlain by an organic layer of varying thickness. Three chambers were installed randomly and fluxes were measured on 23 and 29 June 1994.

North Berwick (NT 555835) - This was an arable agricultural field planted with oilseed rape and an adjacent shelter belt in East Lothian, S.E. Scotland (60m asl), on a brown forest soil with gleying of the Kilmarnock association (Brown & Shipley, 1982). Fluxes were measured 5 times between 8 and 23 June 1995. Nine chambers were installed randomly in the oilseed rape field and three in the shelter belt.

East Linton (NT 613767) - This was an arable agricultural field in East Lothian, S.E. Scotland (50m asl), on a brown forest soil of the Winton series (Brown & Shipley, 1982); the textural class was a sandy clay loam. Fluxes were measured seven times between I4 April and 9 May 1994, before and after the second application of fertiliser (43 kg NH_4NO_3-N ha^{-1}) to a winter wheat crop. On measurement days 14 and 24 April, and 5 May three chambers were measured, on the other days nine chambers were measured.

Dunslair (NT 288436) - This site was in a mixed upland coniferous forest near Peebles, S.E. Scotland. Sites were chosen below and above the altitude which commonly experiences hill cloud, at 380 m asl on a mature sitka spruce plantation on a brown forest soil and at the hill summit at 615 m asl, a mature sitka spruce plantation and a heather moorland on a peaty podzol of the Ettrick association (Brown & Shipley, 1982). The soils at all sites were overlain by at least 10 cm of peat. Three chambers were installed randomly in each plot and fluxes were measured on 27 April and 6 July 1994.

Caithness - (ND 065453). Here the sites included an area of blanket bog and plantations of mature sitka spruce and lodgepole pine at Loch More, Caithness, N.E. Scotland (135 m asl). Nine chambers were installed randomly in the blanket bog, fluxes were measured seven times between 30 May and 10 June 1994. In the forest plots three chambers were installed

randomly in each plot, flux measurements were made on four occasions between 30 May and 10 June.

Springfield Farm - This site was in an area of drained blanket bog near Penicuik, Midlothian, S.E. Scotland. Three chambers were installed and measurements were made on 3 and 25 May 1995.

3.3.2. Field Measurements

Field measurements were made using the static chamber method as described in Chapter 2. Soil samples were analysed for water content, temperature, available NO_3-N and NH_4-N, bulk density and pH as described in Chapter 2.

3.3.3. Statistical analysis

An analysis of variance (ANOVA) was used to determine the significance ($p<0.05$) of differences between CH_4 flux and environmental variables between sites. The relationship between CH_4 flux rates and the environmental variables measured was analysed using Pearson product moment correlation and simple linear regression.

3.4. Results

Methane fluxes and the major soil variables: water content, temperature, bulk density, pH, NO_3-N and NH_4-N, for all sites are summarised in Table 3.1. Fluxes ranged from -12.3 to 6.8 ng m^{-2} s^{-1}. The largest oxidation rates were observed in the mineral forest soils at Glencorse. Both CH_4 emission and oxidation were observed in the peat soils with the largest emission occurring in peats with the highest soil water content.

3.4.1 CH_4 fluxes from mineral soils

Glencorse CH_4 oxidation rates ranged from -4.8 to -12.3 ng m^{-2} s^{-1} (Table 3.1) on the two dates measured and were found to be significantly ($p<0.05$) lower in the alder plantation than in the other sites at Glencorse. The NO_3-N concentration in the soil from the alder plantation was twice as large (8.11 :g g^{-1} dry soil) as from the other plots and the soil was more acidic. Larger rates of CH_4 oxidation were observed for the sitka and birch plots than the grass plot; however, differences were only significant ($p<0.05$) between the grass and sitka plots. No significant relationship between soil bulk density and CH_4 uptake rate was found, but the lowest oxidation rate was associated with the highest soil bulk density. Measured differences between all other soil variables were not significant ($p>0.05$).

Table 3.1. Methane flux and soil physical and chemical characteristics.

Site	CH_4 flux ($ng\ m^{-2}\ s^{-1}$)	n	Soil water content (% dry wt.)	NO_3-N (:g g^{-1}dry soil)	NH_4-N (:g g^{-1}dry soil)	pH ($CaCl_2$)	Soil temp. (°C)	Soil bulk density ($g\ cm^{-3}$)
Mineral soils								
Glencorse grass	-9.8 (1.2)[1]	6	23.3	1.79	5.29	4.7	14.4	1.10
alder	-4.8 (1.9)	6	25.9	8.11	4.48	3.3	11.1	1.13
sitka	-12.3 (1.5)	6	19.5	2.11	3.85	4.2	10.6	1.01
birch	-11.2 (1.6)	6	26.0	3.06	5.46	3.9	11.6	1.11
Devilla pine	-9.9 (4.1)	6	28.9	2.54	3.47	3.1	10.2	1.00
East Linton agricultural	-0.2 (0.6)	45	23.0	11.3	8.6	6.1	11.3	1.23
North Berwick arable	-1.4 (0.2)	45	19.9	1.6	16.4	5.1	10.7	1.30
shelter belt	-6.9 (2.0)	15	29.7	0.3	19.2	4.6	10.4	1.02
Peat Soils								
Dunslair moorland	-6.8 (3.2)	6	147	1.20	6.32	3.5	7.3	nd
high altitude forest	-2.6 (1.0)	6	134	1.42	24.8	3.2	7.5	nd
how altitude forest	-4.6 (2.3)	6	103	1.19	4.91	3.1	9.1	nd
Loch More sitka	6.8 (6.1)	9	516	nd	nd	2.6	8.1	nd
pine 1	4.9 (3.8)	12	654	nd	nd	2.7	7.8	nd
pine 2	-2.1 (1.9)	11	319	nd	nd	2.7	8.3	nd
blanket peat	6.7 (10.9)	40	564	nd	nd	3.2	9.0	nd
Springfield Farm blanket peat	3.9 (6.9)	6	400	nd	nd	2.6	10.5	nd

[1] = mean (standard deviation)
n = number of measurements
nd = not determined

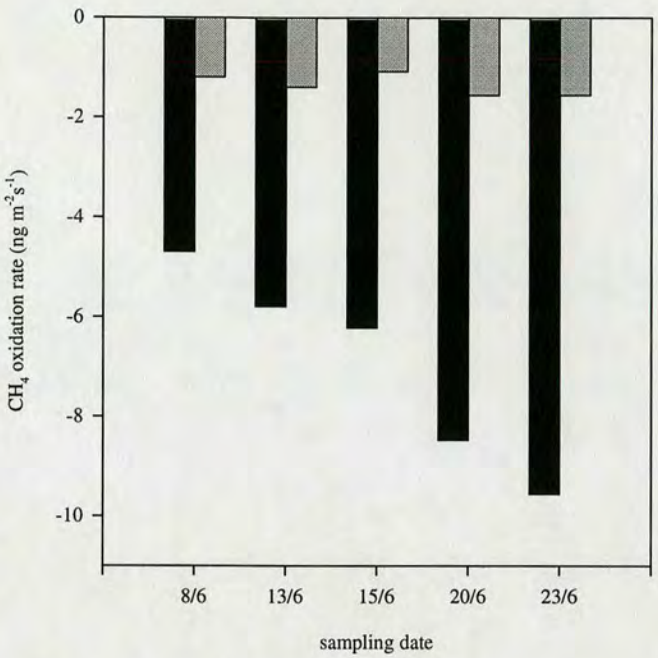

Figure 3.1 Methane oxidation rates at North Berwick, from the oilseed rape field (▣), and adjacent shelter belt (■).

Devilla CH_4 oxidation rates at Devilla forest averaged -9.9 ng m^{-2} s^{-1} on the two dates measured and were similar to the fluxes measured at Glencorse.

North Berwick In the oilseed rape field CH_4 oxidation rates were significantly ($p<0.05$) lower than those measured in the shelter belt (Figure 3.1). Oxidation rates ranged from -1.1 to -1.6 ng m^{-2} s^{-1} compared to -4.7 to -9.6 ng m^{-2} s^{-1} in the shelter belt. CH_4 oxidation rates increased over the measurement period and were correlated ($p<0.05$) with decreasing soil water content and increasing soil temperature. The shelter belt soil responded more positively to changing environmental conditions, with oxidation rates more than doubling compared to only a ~ 30% increase in the oilseed rape field. This was despite the soil in the rape field drying out at a much higher rate. Soil water content was larger and soil bulk density smaller in the shelter belt than in the oilseed rape field. The soil in the shelter belt was also more acidic. Soil NO_3-N concentrations were larger in the oilseed rape field relative to the shelter belt, whereas soil NH_4-N concentrations were larger in the shelter belt, although this difference was not significant ($p>0.05$).

East Linton At East Linton, CH_4 fluxes were measured before and after the second application of N fertiliser (Figure 3.2). The largest CH_4 oxidation rates (-0.7 ng m^{-2} s^{-1}) were

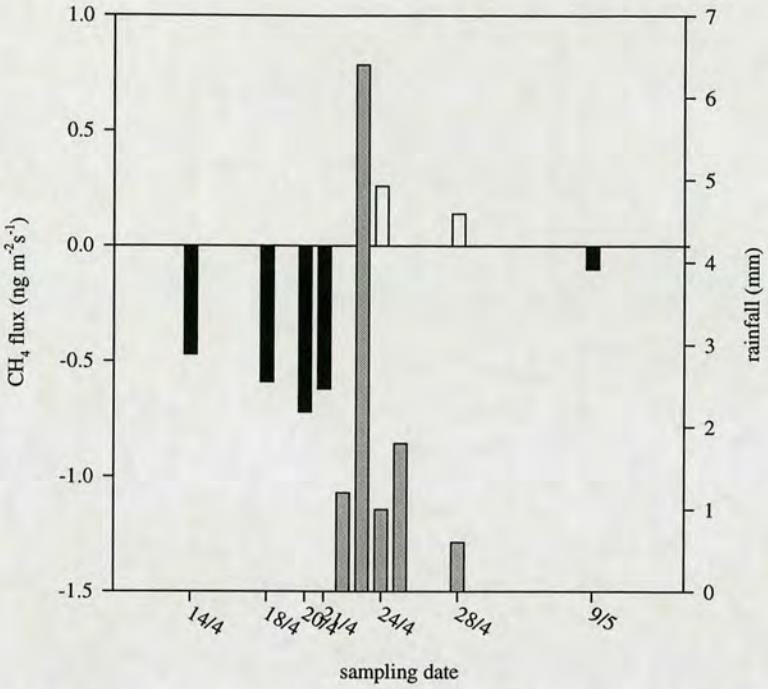

Figure 3.2 CH_4 flux (□ oxidation, ■ emission) and rainfall (▩) (20 April to 29 April) from the agricultural site, East Linton, before and after the application of 43 kg NH_4NO_3-N ha^{-1} on 19 April.

observed before and until one day after fertiliser application when high rainfall transported the fertiliser into the soil and CH_4 emission was observed (0.3 ng m^{-2} s^{-1}). Net oxidation was again observed 16 days after fertiliser application, although rates were six times lower than before fertiliser application (-0.1 ng m^{-2} s^{-1}) and the soil water content was 5% lower.

3.4.2 CH_4 fluxes from peat soils

Dunslair Heights Mean CH_4 oxidation rates ranged from -2.6 to -6.8 ng m^{-2} s^{-1}. A similar pattern of CH_4 oxidation was observed on 27 April and 6 July, with the smallest oxidation rates being found at the high altitude forest site and the largest oxidation rates at the high altitude moor despite the large differences in soil temperature and water content between the two sampling dates (Figure 3.3). The high altitude forest site had significantly smaller oxidation rates ($p<0.05$) on 6 July (high altitude forest -2.9 ng m^{-2} s^{-1}, compared to -9.1 ng m^{-2} s^{-1} and -6.5 ng m^{-2} s^{-1} for the moor and low altitude forest sites, respectively) but not on 27 April. Significantly larger concentrations of soil available NH_4-N were found at the high altitude forest site (24.8 :g NH_4-N g^{-1} dry soil) compared to the low altitude forest and moor sites (4.9 and 6.3 :g NH_4-N g^{-1} dry soil respectively). Soil water content was ~10% higher and the pH was 0.3 units higher at the moor site than at the high altitude forest site. The low altitude forest site had the lowest soil water content, highest soil temperature, and lowest soil N concentrations.

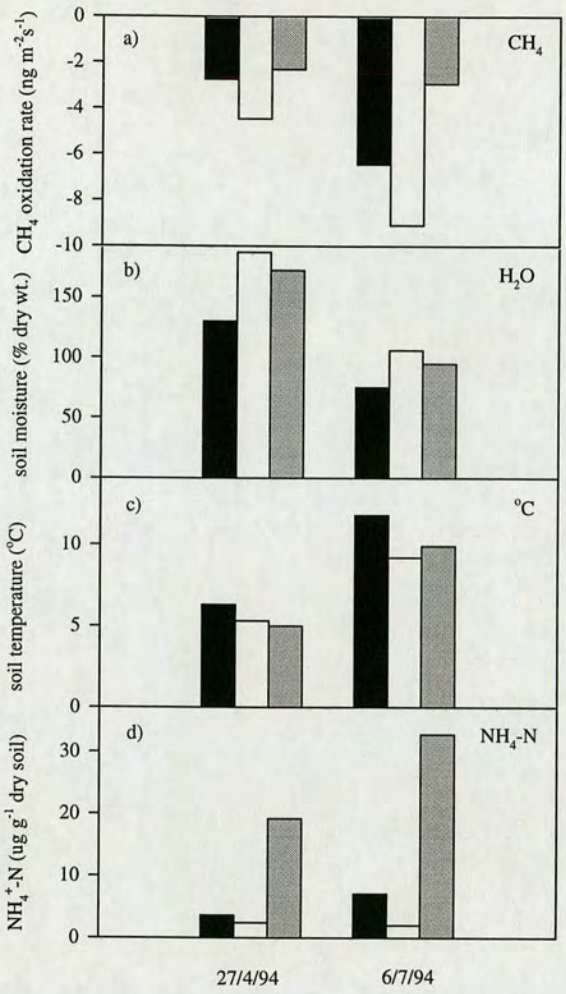

Figure. 3.3 a) CH_4 oxidation at Dunslair on 27/4 and 6/7, **b)** soil water content (% dry weight), **c)** soil temperature (°C) and **d)** available NH_4-N :g g^{-1} dry soil at the (■) low altitude forest, (▩) high altitude forest and (□) moorland.

A large increase in CH_4 oxidation rate was observed between the sampling dates, coinciding with an approximate 50% decrease in soil water content and 50% increase in soil temperature. Oxidation rates at the low altitude forest site increased from -2.7 to -6.5 ng m^{-2} s^{-1} and at the moor site from -4.5 to -9.1 ng m^{-2} s^{-1}. However, at the high altitude forest site only a small ~0.5 ng m^{-2} s^{-1} increase was observed, despite the similar observed change in environmental conditions.

Loch More At Loch More both emission and oxidation were observed. A mean net emission flux of 6.7 ng m^{-2} s^{-1} was measured for the blanket peat, and fluxes ranged from 6.8 to -2.1 ng m^{-2} s^{-1} in the forested plots (Figure 3.4). CH_4 oxidation was only observed in the driest

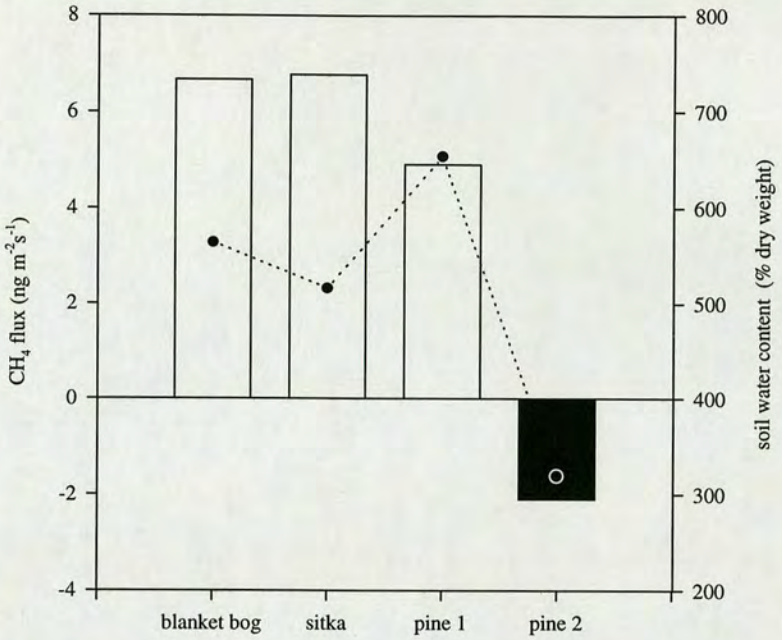

Figure 3.4. Methane flux (□ oxidation, ■ emission) and soil water content (●) from areas of forested and non-forested blanket bog, Loch More, Caithness.

peat. The pH of the blanket peat was significantly less acidic than at any of the forested sites (Table 3.1).

Springfield Farm At Springfield Farm a mean net CH_4 emission of 3.9 ng m^{-2} s^{-1} was observed. The peat had a large water content and was very acidic (Table 3.1).

3.5 Discussion

The results of the field measurements over a range of soils provide examples of both CH_4 emission and oxidation and are within the range of fluxes reported in the literature (Crill, 1991, Dorr *et al.*, 1993, Lessard *et al.*, 1994). The mineral forest soils oxidised CH_4 with fluxes averaging -9.0 ng m^{-2} s^{-1}. In contrast, the agricultural soils showed much smaller rates of CH_4 oxidation and at East Linton emitted CH_4 after prolonged rainfall and fertilisation. The peats showed both oxidation and emission in the range -6.8 to 6.8 ng m^{-2} s^{-1} with the net flux being regulated by soil water content.

To illustrate the range of fluxes at each of the measured sites and summarise the association of CH_4 oxidation and emission fluxes with small and large N input and increasing soil water content, the mean fluxes from each site have been plotted in order of decreasing CH_4 oxidation rates in Figure 3.5. Although the absolute range of fluxes is small,

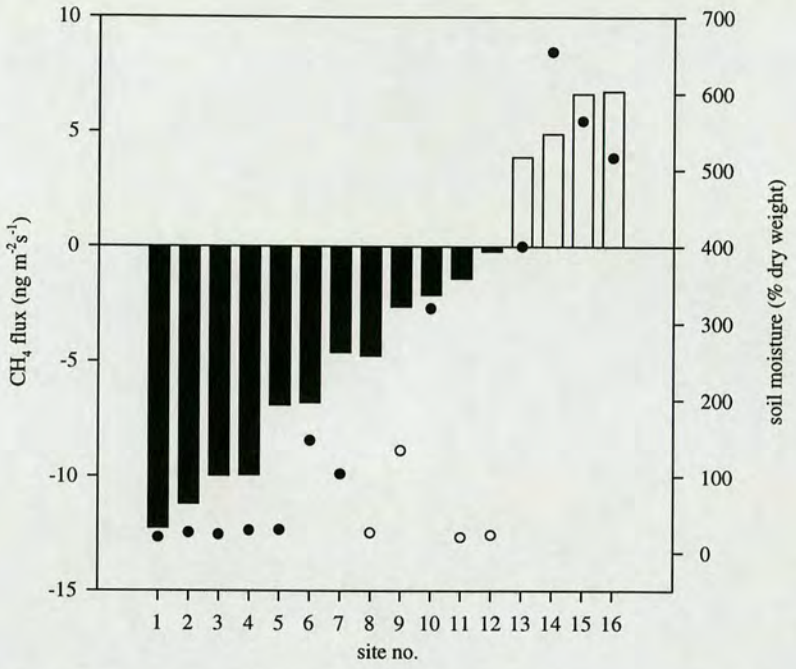

Figure 3.5. CH_4 oxidation (□) and emission (■) rates, and soil water content (% dry weight) from low N (M) and high N (Φ) forest, moorland and agricultural soils. Site 1=Glencorse sitka, 2=Glencorse birch, 3=Glencorse grass, 4=Devilla pine, 5=Dunslair moor, 6=N. Berwick shelter belt, 7=Glencorse alder, 8=Dunslair low altitude forest, 9=Dunslair high altitude forest, 10=Loch More pine 2, 11=N. Berwick arable, 12=East Linton arable, 13=Springfield Farm blanket peat, 14=Loch More Pine 1, 15=Loch More blanket peat and 16=Loch More Sitka. High N sites received N through N deposition, fertilisation and fixation.

and for the CH_4 oxidation rates, which constitute the majority of the data, the overall mean flux and standard deviation are even smaller being -6.1∀ 4.1 ng m^{-2} s^{-1}, there are clear effects of land use and N input associated with the decreased fluxes. It is convenient therefore to discuss the effects of each of the major variables on rates of CH_4 oxidation or emission in turn.

3.5.1 Effect of soil water content

The response of CH_4 oxidation to soil water content has been widely reported for laboratory studies and field observations with a clear inverse relationship between CH_4 oxidation and soil water content (Steudler *et al.*, 1989, Whalen *et al.*, 1990, Whalen *et al.*, 1991). The range in CH_4 flux over peat soils, from emission of 6.8 to oxidation of -6.8 ng $m^{-2}s^{-1}$, coresponds to a wide span in soil water content, 654 to 103 % dry weight. The data plotted in Figure 3.6 show a clear relationship between CH_4 flux and soil water content (r^2=0.836), with a change from oxidation to emission at a soil water content of 325% (∀80% from 95% confidence interval). The effects are consistent with the literature on net exchange of CH_4

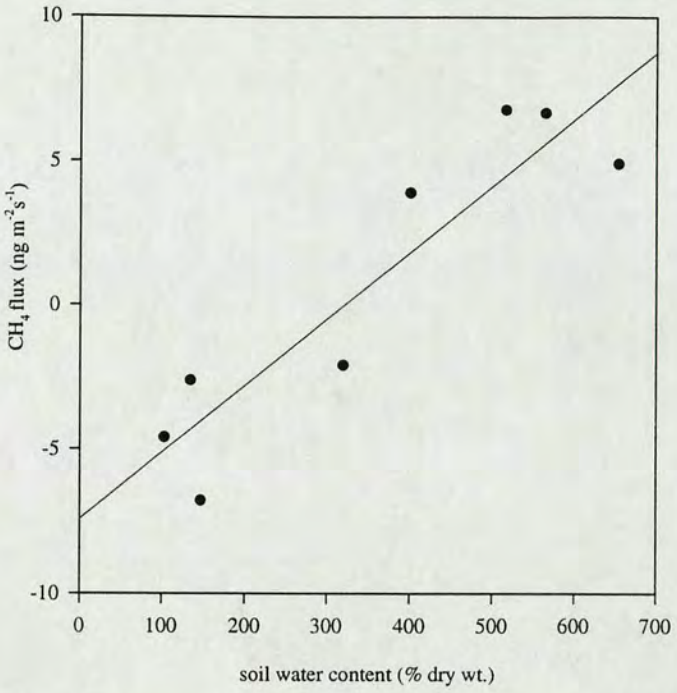

Figure 3.6. The relationship between CH_4 flux from the peat soils studied and soil water content (r^2=0.836, $p<0.05$). The regression equation was; CH_4 flux (ng m^{-2} s^{-1}) = 0.023×%H_2O(dry weight)-7.4

over peatlands (Whalen & Reeburgh, 1990) and show the competition between methanogenesis and CH_4 oxidation as soils become progressively drier. The progression from oxidation in dry soils to emission in wet soils is also closely coupled with oxygen supply and gas transport into the soil. As gaseous diffusion becomes progressively restricted by water filled pore space, the supply of oxygen becomes limited and the redox potential drops, allowing methanogenesis to begin.

3.5.2 Effect of bulk density

Gaseous diffusion in soils is also influenced by soil bulk density. In the measurement of CH_4 oxidation rates in the mineral soils, a negative relationship with soil bulk density was observed (Figure 3.7). Bulk density is a likely indicator of the effect of diffusivity in the soil which influences the gas transport of substrate to methane oxidisers. The two smallest oxidation rates had the largest bulk densities and were found in the agricultural soils. Compaction during cultivation may increase bulk density; Hansen *et al.* (1993) found a 52% reduction in oxidation rates following compaction by tractor traffic. Studies have attributed variations in CH_4 oxidation rates to soil physical parameters; and soil diffusivity has been shown to be the controlling parameter on flux rates in several studies (Dorr, 1993, Keller & Reiners, 1994). Whalen & Reeburgh (1990), however, concluded that CH_4 oxidation was

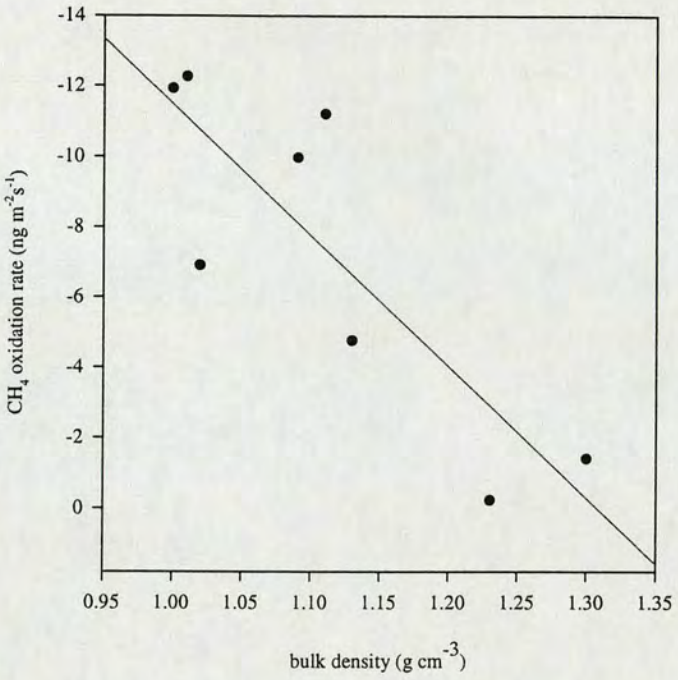

Figure 3.7. The relationship between CH_4 oxidation rates from the mineral soils soil bulk density (r^2=0.711, $p<0.05$). The regression equation was CH_4 flux (ng m^{-2} s^{-1}) = 37.35×bulk density(g cm^{-3})-48.8.

not controlled by gas diffusion in tundra soils, and Crill (1991) found a diffusional control on oxidation only during periods of most active microbial activity.

3.5.3. Effects of Nitrogen

Increased N loading due to N fixation, atmospheric deposition of N, and fertilisation were associated with decreases in the soil sink for CH_4 on a range of soil types and land uses. At all sites studied, the significance of the correlation between CH_4 flux, and soil water content increased when the sites with high N inputs were excluded, indicating the importance on N as a mediator of CH_4 flux rates (Figure 3.5).

Inhibition of CH_4 oxidation in soils by various forms of N has been documented for many soils (Steudler *et al.*, 1989, Adamsen & King, 1993, Castro *et al.*, 1994). Experiments on pure cultures have demonstrated the inhibitory effects of NH_3 on CH_4 oxidation, and of CH_4 on NH_4^+ oxidation (O'Neill & Wilkinson, 1977, Hyman & Wood, 1983). This inhibition is thought to be due to competitive inhibition for active sites on the mono-oxygenase enzyme system (Bedard & Knowles, 1989). However, results are not always consistent with the competitive inhibition theory, as the effects of the inhibition are often long term (Mosier *et al.*, 1991), lasting long after added NH_4^+ has been nitrified.

Methane oxidation rates at Glencorse were found to be significantly lower in the alder plot than in the adjacent birch, sitka and grass plots. The increased available NO_3-N concentrations in the soil caused by N fixation by the actinomycete *Frankia* in the root nodules of red alder (Stettler, 1978) is consistent with the low rate of CH_4 oxidation observed. However, high soil bulk density was also found at this site and would have contributed to the low oxidation rate observed. This soil was also more acidic, although no correlation between soil pH and oxidation rates was found.

At East Linton, the winter wheat field, the mean oxidation rate was only 2% of the rates observed at forest sites on similar soil types, and CH_4 emission was observed after a period of rainfall. It is possible that the effects of N fertilisation, compaction, and cultivation had decreased the capacity of this soil to oxidise CH_4 to an extent that net CH_4 emission, from anaerobic micro-sites, was observed. Compaction, by decreasing the diffusion of oxygen into the soil could have stimulated CH_4 production in anaerobic microsites. Sixteen days after fertilisation, when the soil water content had substantially decreased, CH_4 oxidation rates were six times lower, showing the inhibitory effect of N fertilisation.

At North Berwick an 80% reduction in CH_4 oxidation rates was observed in the oilseed rape field relative to the adjacent shelter belt. Similar results have been found in other studies (Lessard *et al.*, 1994, Dobbie *et al.*, 1996). The observed decrease in oxidation rates in the oilseed rape field as a result of cultivation was a consequence of a number of factors including the inhibitory effects of N. The soil NO_3-N concentrations were larger in the oilseed rape field than in the shelter belt and the soil NH_4-N concentrations were higher in the shelter belt, suggesting lower nitrification rates in the shelter belt relative to the oilseed rape field. However, the effects of N cannot be separated from the increased bulk density, due to compaction, found at this site.

Large soil NO_3-N concentrations were associated with small CH_4 oxidation rates in several of the mineral soils studied. No correlation was found between oxidation rates and soil NH_4, which agrees with findings by Crill *et al.* (1994) and Sitaula & Bakken (1993). It has been suggested that nitrification rates rather than NH_4 concentrations may be responsible for the inhibition (Mosier *et al.*, 1991, Sitaula & Bakken, 1993). The high soil NO_3-N concentrations (the end product of nitrification) in this study may be indicative of nitrification rates and therefore the inhibition of CH_4 oxidation rates could be related to N turnover. However, the mechanism of the inhibition is not certain.

At Dunslair Heights, the smallest CH_4 oxidation rates were found at the forested site on the hill summit, which was the site with the largest soil available NH_4-N concentration,

compared to the low altitude forest and the adjacent moorland site. The hill summit is frequently in cloud and receives much larger inputs of deposited N species. The total annual deposition of N at the site increases from 6.4 kg ha^{-1} at 275 m to ~ 24.3 kg ha^{-1} at 615 m, the summit (Crossley *et al.*, 1992). Because the trees intercept polluted cloud water, HNO_3 and NH_3 deposition rates were up to 90% larger at the afforested high altitude site (Fowler *et al.*, 1989), resulting in the much higher soil available NH_4-N concentrations observed relative to the adjacent moorland site. The change in soil water content and temperature that occurred over the study period did not significantly increase oxidation rates at the high altitude forest site, indicating that N dynamics were possibly having a negative effect on the microbial population response to changes in soil water content and temperature. Environmental variables measured, such as soil water content, temperature and pH, did not appear to correlate with the observed differences in CH_4 oxidation rates.

In contrast to the mineral soils, low rates of CH_4 oxidation at Dunslair were associated with high soil NH_4-N concentrations and NH_3 may have been acting as a competitive inhibitor of CH_4 oxidation rates (Bedard & Knowles, 1989). It is also possible that an ion toxicity mechanism due to the high atmospheric pollution loading at this site was having an inhibitory effect on methanotrophs and nitrifiers. Several studies have identified salts (Adamsen & King, 1993) and the products of NH_4^+ oxidation, hydroxylamine and nitrite, as having toxic effects on methanotrophs (Schnell & King, 1994). More work is required to estimate the relative effects of deposited species on CH_4 oxidation rates.

3.5.4. Effects of temperature and pH

The temperature response of CH_4 oxidation has been reported for a variety of soil environments, with Q_{10} values in the range 1.3 to 2.3 (Whalen *et al.*, 1990, Dunfield *et al.*, 1993, Adamsen & King, 1993). No significant correlation with temperature was observed in this study; however, the temperature range in which fluxes were measured was small, averaging 9.9 ∀ 1.9 °C.

The effect of pH on CH_4 oxidation rates is not well documented. Hητsch *et al.* (1994) observed an inhibitory effect of low pH on CH_4 oxidation. Dunfield *et al.* (1993) concluded that the microorganisms involved in CH_4 metabolism in peat soils were not well adapted to low pH values, although studies including this one have measured oxidation in soils of low pH (Castro *et al.*, 1993). The acidifying properties of conifers is well documented (Hornung, 1985) and low pH values were associated with soils under the conifer plantations at Dunslair Heights (pH 3.2) and Loch More (pH 2.7). A low pH was

observed in the alder plot at Glencorse (pH 3.3); red alder is also known to accelerate soil acidification (Sheppard, 1993). However, overall no correlation was observed between CH_4 oxidation rates and soil pH at the sites studied.

3.5.5. Effects of disturbance and cultivation

The effect of land use was well illustrated by the small fluxes found at East Linton and the large difference in the oxidation rates between the oilseed rape field and shelter belt at North Berwick. The decrease in CH_4 oxidation rates associated with cultivation and disturbances, such as the conversion of forest to agriculture, have been reported in several studies (Keller *et al.*, 1990, Lessard *et al.*, 1994, Dobbie *et al.*, 1996). A number of factors may contribute to the observed decrease in CH_4 oxidation rates. N fertilisation effects have been well documented (Steudler *et al.*, 1989, Castro *et al.*, 1994), and increased compaction due to cultivation, leading to a decrease in soil diffusivity, has also been shown to have a negative effect on CH_4 oxidation (Hansen *et al.*, 1993). Bender & Conrad (1994) suggested that the vertical stratification of CH_4 oxidising activity was destroyed by ploughing, influencing oxidation rates, and Priemé *et al.*, (1997) suggested that the inhibition was a result of changes in microbial numbers or enzyme activity as well as changes in the diffusivity status of the soil.

The birch and sitka plots at Glencorse had higher CH_4 oxidation rates than the grass plot, although the difference was only significant for the sitka plot which also had a lower soil water content. It would therefore be premature to speculate on the effects of afforestation on CH_4 oxidation rates at this site. Priemé *et al.*, (1997) observed recovery times of CH_4 oxidation following disturbance to be greater than 100 years, therefore it is unlikely in this soil that any recovery, over such a short time period, would have occurred. Possible reasons for higher CH_4 oxidation rates under trees include a decrease in soil bulk density and formation of a stable soil structure (Brady, 1990), leading to improved aeration. Keller *et al.* (1990) speculated that soils under the forest canopy reside in a more stable micro-climate where soil water content and temperature are well buffered. In the grassland it is possible that the dense rhizosphere and associated high microbial activity results in competition for O_2, which leads to the formation of anaerobic microsites and hence lower oxidation rates.

3.6 Summary

Comparing CH_4 flux rates and soil environmental parameters from a wide range of soil environments can provide an insight into the controls on CH_4 flux rates. Field measurements of CH_4 flux from a range of soil environments showed that soil water content, N input and soil diffusivity strongly influenced CH_4 flux. Both CH_4 oxidation and emission were observed with fluxes ranging from -12.3 to 6.8 ng m^{-2} s^{-1}. In the peat soils, soil water content was found to be the most important environmental variable affecting the magnitude and direction of flux, with CH_4 oxidation being observed below 325% (± 80%), and net emission occurring at soil water contents above this value. The importance of soil water content in determining the magnitude and direction of flux from these environments demonstrates the sensitivity of peatlands to future changes in climate. Increased N loading due to atmospheric deposition, and scavenging by trees, was associated with low CH_4 oxidation rates in an upland peaty podzol. The effects of atmospheric pollution could have important mediating effects on the size of the CH_4 soil sink. The mineral soils showed a range of oxidation rates over a small range in soil water content. The highest rates were observed in mineral forest soils, with the exception of the red alder site where high rates of N cycling and a higher soil bulk density were correlated with lower CH_4 uptake rates. The lowest oxidation rate was observed in an agricultural soil where compaction and fertilisation resulted in high soil bulk densities and soil NO_3-N concentrations. Mineral soil oxidation rates were significantly correlated with soil bulk density, indicating the importance of soil diffusivity in determining the size of the soil sink for CH_4.

Chapter Four

The effect of nitrogen deposition and seasonal variability on CH_4 oxidation rates in an upland spruce plantation and moorland.

(A version of this chapter is published in Atmospheric Environment 31: 3693-3706, 1997, as: The effect of nitrogen deposition and seasonal variability on CH_4 oxidation and N_2O emission rates in an upland spruce plantation and moorland, with U. Skiba, L.J. Sheppard, B. Ball, J.D. Roberts, K.A. Smith and D. Fowler as co-authors)

4.1 Abstract

Rates of CH_4 oxidation were measured at upland moorland and coniferous sites in Southern Scotland to investigate seasonal variations in flux and the influence of atmospheric N inputs on the land-atmosphere exchange of CH_4. Rates of CH_4 oxidation ranged from -0.4 to -16.7 ng m^{-2} s^{-1} and showed a strong seasonal response with a summer maxima. Total annual uptake of CH_4 was estimated at 1.8, 0.7 and 1.3 kg ha^{-1} y^{-1} at the moorland, high altitude forest and low altitude forest sites, respectively. Highly significant correlations were observed between CH_4 oxidation rates and soil temperature, with E_a between 60 and 140 kJ mol^{-1}. Soil water content and temperature manipulation experiments confirmed that soil temperature was controlling CH_4 oxidation rates at soil water contents commonly observed in the field. This relationship was attributed to the high soil gas diffusivity of the peat. CH_4 oxidation rates were 46 and 61% smaller at the high altitude forest site than at the adjacent moorland site, or the low altitude forest site, respectively, coincident with a large concentration of soil NH_4-N due to high N deposition at the hill summit. Addition of N as NO_3 to soil cores resulted in a 86% reduction in oxidation rates and addition of N as NH_4Cl in a 70% reduction. However, a 75% reduction in oxidation rates was also observed for cores treated with NaCl. Soil available NH_4-N concentration was not related to the degree of inhibition in CH_4 oxidation rates observed in the soil cores.

4.2. Introduction

Long-term studies of CH_4 oxidation rates are required in order to quantify the seasonal response and to gain a mechanistic understanding of the processes regulating fluxes. Seasonal studies of CH_4 oxidation rates have shown various responses to changes in soil water content and temperature (Crill, 1991, Dorr *et al.*, 1993, Lessard *et al.*, 1994). Several studies have shown a lack of a strong temperature response (King & Adamsen, 1992, Lessard *et al.*, 1994), thought to be a result of a diffusional control on the supply of substrate to methanotrophs (Dorr *et al.*, 1993). Soil water content has been shown to control CH_4 oxidation rates in several studies either by restricting the transport of CH_4 to methanotrophs (Lessard *et al.*, 1994, Castro *et al.*, 1995), by physiological stress at low water potentials (Adamsen & King, 1993), or by shifting the balance between CH_4 production and CH_4 oxidation (Yavitt *et al.*, 1995). However, soil temperature is important in some soils. Castro *et al.* (1995) found a temperature dependence of CH_4 oxidation rates at low temperatures, but concluded that when microbial communities reached their optimum temperature for oxidation, other factors such as soil water content exerted primary control on rates.

Inhibition of CH_4 oxidation rates by various N compounds has been widely documented for a range of ecosystems, although the majority of studies have been field manipulation experiments or laboratory-based experiments on soil microcosms (Steudler *et al.*, 1989, Crill *et al.*, 1994). Competitive inhibition between CH_4 and NH_3 for active sites on the mono-oxygenase enzyme system, which catalyses the initial stage of CH_4 and NH_4 oxidation, has been suggested by Bedard & Knowles (1989) as a mechanism for the observed inhibition. However, conflicting results between soils, effects of amount and type of N applied, and duration of the inhibition (Steudler *et al.*, 1989, Nesbit & Breitenbeck, 1992, Adamsen & King, 1993, Dunfield *et al.*, 1995) question this hypothesis. Nitrogen deposition in temperate and boreal zones of the Northern Hemisphere has been estimated to be about 18 Tg y^{-1} (Melillo *et al.*, 1989). The major sources of nitrogen oxides (NO_x) and ammoniacal N (NH_x) are fossil fuel production and agricultural practices, respectively. N deposition can result in eutrophication or acidification. Forest ecosystems are known to be efficient sinks for atmospheric pollutants, particularly at high altitudes (Fowler *et al.*, 1989 and references therein). The effects of the atmospheric deposition of N on rates of CH_4 oxidation have not been fully investigated and the extent of the inhibition on a regional or global scale is unknown.

In this study the impact of enhanced N deposition on the oxidation of CH_4 was investigated at a high altitude (615 m) conifer forest and moorland site and compared to a

low altitude (380 m) conifer forest. Field measurements were made over a period of 18 months to measure the seasonal variations in CH_4 oxidation rates. In addition, the effects and relative importance of soil water content, temperature and N deposition on CH_4 oxidation rates in this soil were examined using laboratory manipulations of soil columns.

4.3. Materials and Methods

4.3.1. Site Description

Field measurements were made at Dunslair Heights (NT 288436), a mixed upland coniferous forest near Peebles, S.E. Scotland (Plate 4.1). Sites were chosen below and above the altitude which commonly experiences hill cloud. The soils at Dunslair were very varied, which made it impossible to find sites above and below cloudbase identical in soil type and age and type of forest plantation. However, differences were minimised by site selection.

Low forest site (380 m asl) A Sitka spruce plantation on a brown forest soil of the Rowanhill Association (Brown & Shipley, 1982), overlain by at least 10 cm of peat, with a thin litter layer (<2 cm). The surface vegetation was sparse, dominated by *Deschampsia flexuosa.*

Top forest site (615 m asl). This was a Sitka spruce plantation at the hill summit. The soil was a peaty podzol of the Ettrick association (Brown & Shipley, 1982); the litter layer was ~7 cm thick and the peat (10-20 cm) overlay a hard rocky subsoil. The surface vegetation had ~ 75% cover and consisted of *Anthoxanthum odoratum*, *Pleurozium schreberi* and *Vaccinium myatilus.*

Moorland site (615 m asl). This was an area of moorland adjacent to the top forest site. The soil was a peaty podzol of the Ettrick association (Brown & Shipley, 1982); the litter layer was ~10 cm thick and the peat (10-20 cm) overlay a hard rocky subsoil. The surface vegetation had 100% cover and the highest species diversity of the sites investigated, dominated by *Anthoxanthum odoratum* with *Festuca ovina, Galium saxatili, Polytrichum commune*, *Pleurozium schreberi, Rhytidiadelphus squarrosus* and *Vaccinium myatilus.*

4.3.2. Field measurements

Measurements were made using the static chamber method described in Chapter 2. Three chambers were permanently installed at each site and fluxes were measured at three to four weekly intervals from April 1994 to November 1995. Three soil samples (top 10 cm) were taken and bulked from each site, every time fluxes were measured, and analysed for water content, soil available N (NH_4 and NO_3) and pH as described in Chapter 2. Measurements of soil diffusivity were made by Bruce Ball (SAC) using a tracer technique (Ball *et al.*, 1994).

Plate 4.1 The moorland and Sitka spruce plantation at the summit of Dunslair Heights.

4.3.3. Laboratory studies of CH_4 oxidation rates

Laboratory manipulations were carried out using repacked soil columns. Fresh soil from the moorland site (top 20 cm) was transported to the laboratory and air dried, prior to adjustment to the required water content. Three replicate soil columns containing 200 g sub-samples were used per treatment. Columns (5 cm i.d. and length 20 cm) were sealed at both ends with rubber bungs. Ambient air was pumped from a tedlar bag, through an inlet and over the surface of the soil, and was collected in another tedlar bag. Flow rates through each column were measured and averaged 15 ml min^{-1}. Samples were analysed by GC. The experiments were carried out in controlled environment rooms in the dark and at 65% humidity.

4.3.3.1 Soil water content and temperature manipulation

Soil water contents were adjusted to 80, 120, 145, 180 and 210 % (dry weight) by spraying each soil subsample with deionised water. Carefull mixing ensured even soil water content distribution. The soil columns were transported to a controlled environment room where a temperature response experiment was carried out. Soil columns were allowed to equilibrate for 24 hours at each of the following temperatures: 5, 10, 15, 20, 25 °C, before CH_4 oxidation measurements were made.

4.3.3.2 Nitrogen addition experiment

Soil water contents were adjusted to 165 % (dry weight). 40 kg ha^{-1} N as NH_4NO_3, $NaNO_3$

or NH_4Cl and an equivalent molar input of NaCl was added to soil columns (n=3 per treatment) in the deionised water used to adjust the water content. Control columns received deionised water only. The columns were allowed to equilibrate in a controlled environment room at 15°C for 24 hours and before the first measurement was made. CH_4 oxidation rates were measured 5 times between 9 Oct and 24 Oct 1995. N_2O emissions were measured using electron capture gas chromatography (Chrompack CP9000).

4.3.4 Statistical analysis

Relationships between daily mean fluxes and environmental parameters were investigated using the Pearson product moment correlation and simple linear regression. In order to investigate significant differences between sites, CH_4 oxidation rates were split (due to the large seasonal variability) according to the mean soil temperature (7.2 °C) into winter and summer groups. An analysis of variance (ANOVA) was then carried out on both groups.

4.4 Results

4.4.1 Seasonal field measurements of CH_4

Methane oxidation rates showed a strong seasonal dependence, with summer maxima exceeding winter values by an order of magnitude at the moorland and low altitude forest sites (Figure 4.1a & b). Oxidation rates ranged from -0.9 to -16.7 ng m^{-2} s^{-1} at the moorland and -0.4 to -10.4 ng m^{-2} s^{-1} at the low altitude forest site. At the high altitude forest the seasonality was less pronounced (Figure 4.1c), with summer oxidation rates exceeding winter rates by a factor of 6. Oxidation rates ranged from -0.7 to -4.5 ng m^{-2} s^{-1}. A significant positive correlation with soil temperature was found at all sites, temperatures ranged between 0.7 and 13.9 °C. An exponential temperature response (r^2 = 0.766, $p<0.001$) was observed at the moor site (Figure 4.2). At the low and high altitude forest sites the observed response appeared linear. Activation energies (E_a) calculated from Arrhenius plots were similar at the moor and low forest (E_a of 118 and 137 kJ mol^{-1} respectively), but much lower at the high altitude forest site (E_a of 58 kJ mol^{-1}). The Arrhenius plot comparing the low and high altitude forest sites is shown in Figure 4.3. Q_{10} values were calculated using the following equation (Winkler *et al.*, 1996):

$$Q_{10} = \exp E_a/R(1/T+10 - 1/T) \qquad (1)$$

Where E_a = activation energy, T = temperature (K) and R = universal gas constant. $Q_{10(5\text{-}15°C)}$ values were 7.8, 5.9 and 2.4 for the moorland, low altitude forest and high altitude forest sites, respectively.

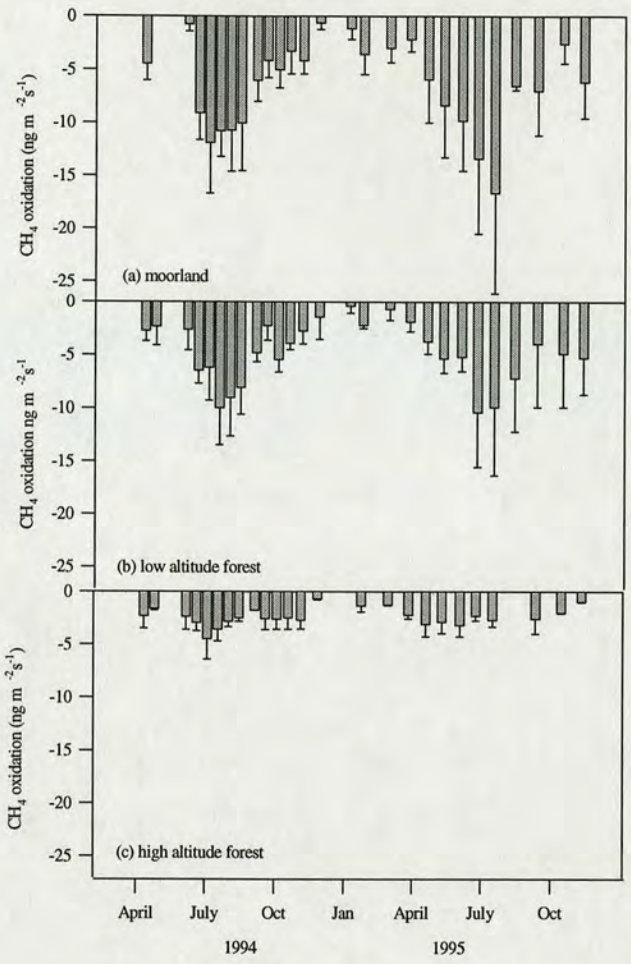

Figure 4.1. Seasonal variation in CH_4 oxidation rates from **a)** the moorland site (615 m), **b)** the low altitude forest site (380 m), and **c)** the high altitude forest site (615 m) between April 1994 and November 1995. Error bars are the standard deviation from the mean.

Methane oxidation rates were only significantly correlated with soil water content at the low altitude forest site (r^2=0.372, $p<0.001$); however, soil water content and temperature were also significantly correlated ($p<0.05$). Mean soil temperature and total rainfall data 24 hours before flux measurement, are shown in Figure 4.4. There was no apparent seasonal cycle in soil water content at the hill summit, possibly due to high spatial variability, reflected in the absence of a correlation between soil water content and rainfall. However, weak correlations between total rainfall 24 hours before the measurement was made and CH_4 oxidation rates were observed ($p<0.05$) for all sites. Rates of CH_4 oxidation were also positively correlated with available NH_4-N concentrations in the soil at the low altitude forest and the moor sites ($p<0.05$).

The significance ($p<0.05$) of the differences between sites varied in response to season. During the summer period CH_4 oxidation rates at the moorland and low altitude forest were not significantly different, but were significantly larger than oxidation rates at

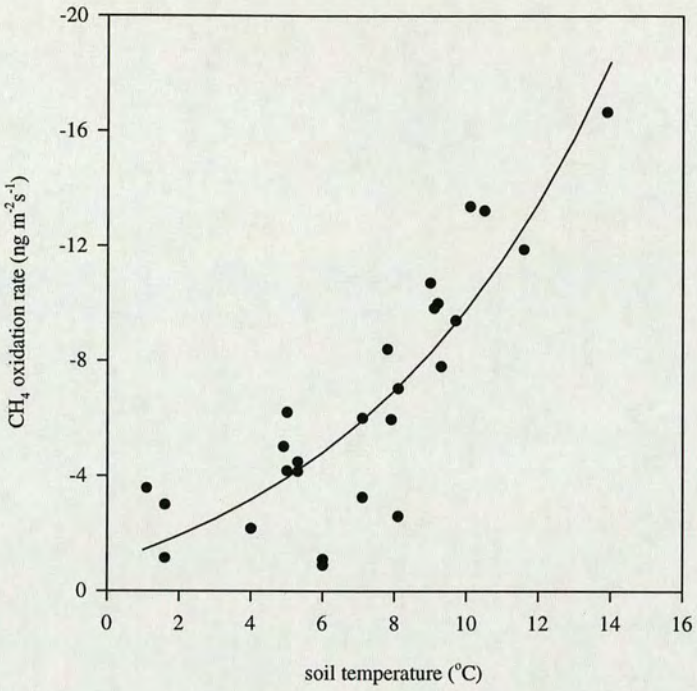

Figure 4.2. The temperature response of CH_4 oxidation rates at the moorland site. An exponential curve was fitted through the data. The regression equation was: CH_4 oxidation rate (ng m^{-2} s^{-1}) = 3.0×exp0.14×temperature (°C) - 2.0 ($r^2 = 0.766$, $p<0.001$).

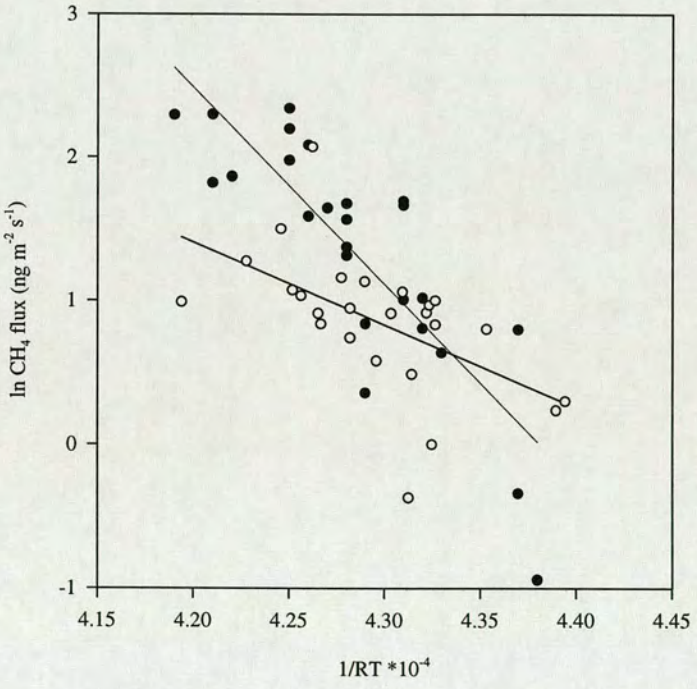

Figure 4.3 Arrhenius plot of the temperature response at the low (●) and high (○) altitude forest sites. 1/RT = the reciprocal of absolute temperature × the ideal gas constant R.

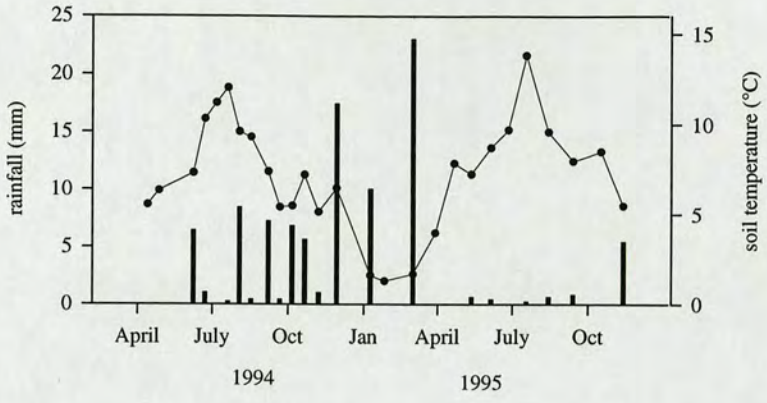

Figure 4.4. Mean soil temperature (●) and total rainfall (■), 24 hours prior to CH_4 measurement throughout 1994/95.

Table 4.1 An estimate of the annual CH_4 oxidation rate for the period 1 Aug 1994 to 1 Aug 1995.

	forest at 380 m	forest at 615 m	moorland at 615 m
CH_4 oxidation rate ($kg\ ha^{-1}\ y^{-1}$)	1.3	0.7	1.8

the high altitude forest site. During the winter CH_4 oxidation rates at the forested sites were not significantly different but oxidation rates at the moorland site were still significantly larger than those at the high altitude forest site. The small oxidation rate at the high altitude forest coincided with an increase in N deposition and consequently large soil available NH_4-N concentrations. A comparison of the periods April to November 1994 and 1995 suggests that differences between years were not significant (Table 4.2). This was true for the mean flux of 13 sampling occasions in 1994 (April 26th to November 21st) and 10 sampling occasions in 1995 (April 12th to November 27th).

Annual rates of CH_4 oxidation at the high altitude forest site were 46 and 61% lower than at the adjacent moorland site, or the low altitude forest site, respectively. The integrated flux was calculated from the running mean of adjacent measuring periods, which was then multiplied by the number of days between measurements (Table 4.1).

Table 4.2. Comparison of average CH_4 oxidation rates, soil temperature and water content for the period 26 April - 21 Nov 1994 and 12 April - 27 Nov 1995

	1994	1995
CH_4 oxidation (ng m^{-2} s^{-1})		
forest at 380 m	-5.1 (3.1)	-5.7 (4.3)
forest at 615 m	-2.7 (1.1)	-2.5 (0.9)
moorland at 615 m	-6.7 (4.2)	-7.9 (6.0)
Soil water content (% dry weight)		
forest at 380 m	111 (23)	93 (18)
forest at 615 m	157 (44)	162 (32)
moorland at 615 m	143 (30)	129 (27)
Soil temperature (°C)		
forest at 380 m	8.6 (2.6)	8.7 (2.4)
forest at 615 m	7.5 (2.2)	7.8 (2.8)
moorland at 615 m	7.5 (2.2)	8.4 (2.7)

Data are means, with standard deviations shown in brackets, of 35 to 38 measurements at 15 sampling dates in 1994 and of 26 to 29 measurements at 10 sampling dates in 1995.

4.4.2 Soil parameters

Available NH_4-N concentrations in the soil were found to be significantly ($p<0.05$) larger at the hill summit than below cloudbase. The largest NH_4-N concentrations were found at the high altitude forest site, averaging 17.0 :g g^{-1} dry soil, compared to that found at the adjacent moorland site and the low altitude forest site, which had 10.6 and 5.6 :g g^{-1} dry soil, respectively (Table 4.3). The concentration differences were very consistent with the data for N deposition at Dunslair (Table 4.3) where the annual N deposition at the moorland site at the hill summit was almost 4 times larger than an open site below cloudbase (Crossley *et al.*, 1992). N deposition rates to the forest canopy are likely to be up to 90% larger than those measured at the open sites, due to the efficient interception of cloudwater by the trees (Fowler *et al.*, 1989). Available NO_3-N in the soil was not found to be significantly different between sites. The soil was more acidic at both the forested sites than at the moorland site. It was usually warmer (~1 °C) at the low altitude forest site than at either site on the hill summit. The low altitude forest soil was frequently drier than the high altitude sites

Table 4.3. Soil characteristics measured at Dunslair over the measurement period 27 April 1994 to 27 Nov 1995.

	Forest, 380 m	Forest, 615 m	Moorland, 615 m
NO_3-N (ug g^{-1} dry soil)	0.8 (1.0)[a]	1.7 (3.9)	0.7 (0.9)
NH_4-N (ug g^{-1} dry soil)	5.6 (3.0)[a]	17.0 (5.0)	10.6 (5.8)
N deposition (kg ha^{-1} y^{-1})	6.4[b]	~46.2[c]	24.3
pH ($CaCl_2$)	3.1	3.2	3.3
soil diffusivity (mm^{-2} s^{-1})	5.14 (1.69)	5.28 (1.25)	6.83 (2.20)

[a] mean (standard deviation) n = 20
[b] data from Crossley *et al.*, (1992)
[c] scaled up from Fowler *et al.*, (1989)

($p<0.001$) and differences between the forest and moorland sites at the summit were significant at $p<0.05$ (Table 4.2). The largest soil gas diffusivity was found at the moorland site, 6.83 mm^{-2} s^{-1}. The low and high altitude forested sites had similar gas diffusivities of about 5.20 mm^{-2} s^{-1}.

4.3 Soil water content and temperature manipulation

Soil water content did not significantly ($p<0.05$) influence oxidation rates in the range 80 to 180 % (dry weight) at any temperature between 5 and 25 °C. At the largest soil water content (210%) CH_4 oxidation rates were significantly lower at all temperatures (Figure 4.5). Significant temperature responses were observed for columns with soil moistures between 80 and 180 %, with mean oxidation rates increasing from 5.6 ng m^{-2} s^{-1} at 5 °C, to 17.2 ng m^{-2} s^{-1} at 20 °C. The temperature response for the cores at 210% was smaller, with oxidation rates increasing from 0 at 5 °C to 3.5 ng m^{-2} s^{-1} at 20 °C. The temperature response observed in the laboratory compared well with the temperature response observed in the field (Figure 4.6). The optimum temperature, for CH_4 oxidation, was about 20 °C.

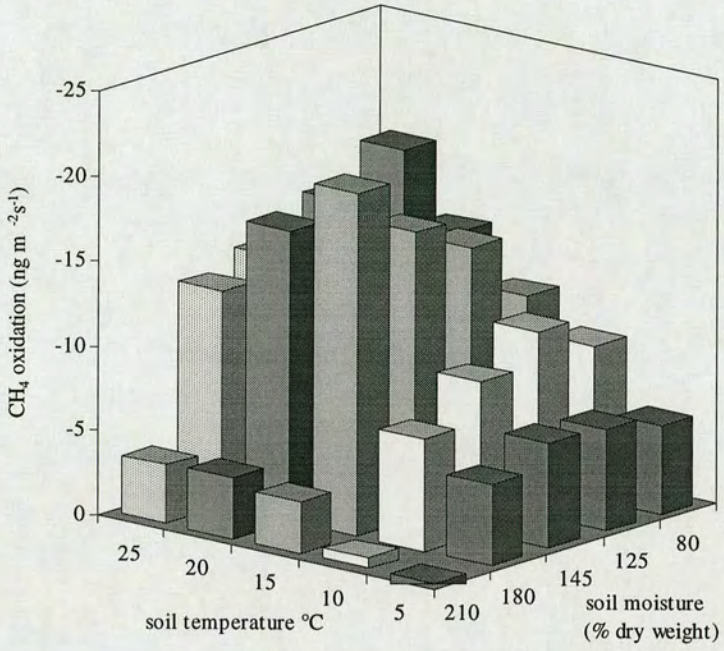

Figure 4.5. The soil water content and temperature response of CH_4 oxidation rates in soil cores under controlled conditions from the moorland site.

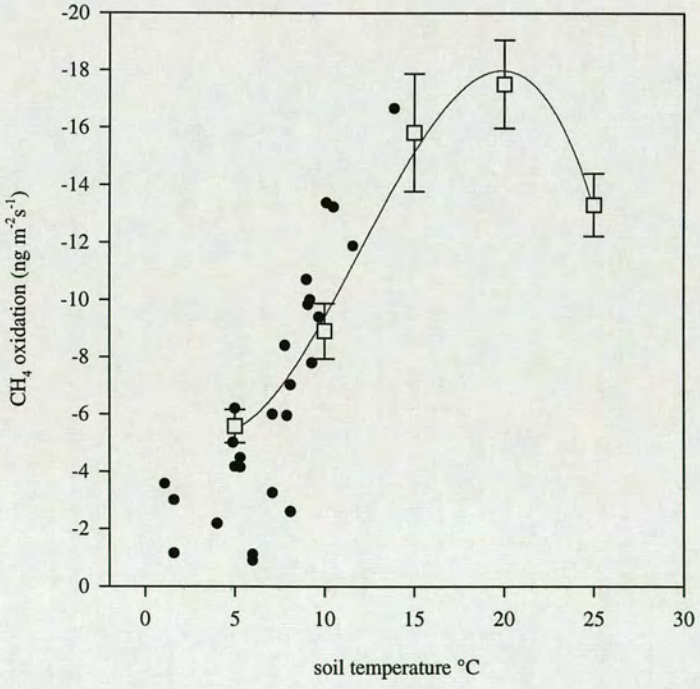

Figure 4.6. The temperature response of CH_4 oxidation rates from soil columns (between 5 and 25 °C) under controlled conditions in the laboratory (□), compared with field measurements (●) of the temperature response at the moorland site. A second-order polynomial curve was fitted to the laboratory data.

Table 4.4. Soil available NH_4-N and NO_3-N concentrations (:g g^{-1} dry soil) for each measurement day of the N addition experiment.

	CONTROL		NH_4NO_3		$NaNO_3$		NH_4Cl		NaCl	
date	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
10/11	0.8	5.6	15.3	14.5	25.7	6.8	0.8	28.1	0.8	5.6
11/11	0.0	4.8	16.5	14.1	28.9	6.0	0.8	22.9	-	-
13/11	-	-	16.9	15.3	27.3	6.4	1.2	26.5	1.2	6.4
17/11	0.8	5.2	17.3	17.7	26.9	6.8	2.0	22.5	1.6	4.8
23/11	0.8	4.8	18.5	12.9	26.5	4.8	3.2	19.7	2.0	4.4

4.4.4 Nitrogen addition experiment

Additions of the various N compounds and NaCl resulted in a large significant decrease in CH_4 oxidation rates (Figure 4.7). In the NH_4NO_3 and the $NaNO_3$ treated cores oxidation rates were reduced by 87% and 86%, whereas for the NH_4Cl and NaCl treated cores, oxidation rates were reduced by 70% and 75%, respectively. CH_4 oxidation rates for each treatment did not differ significantly throughout the 14 day measurement period, indicating that the observed inhibition had occurred within the first 24 hours of adding the treatments. Soil available N concentrations over the measurement period are shown in Table 4.4. The degree of inhibition in CH_4 oxidation rates observed did not correlate with the soil available NH_4-N concentrations. But soil available NO_3-N concentrations were largest in the NH_4NO_3 and $NaNO_3$ treated columns and coincident with the largest inhibition of CH_4 oxidation (Figure 4.7). Additions of NH_4NO_3 and $NaNO_3$ increased the N_2O emissions by up to an order of magnitude. Over the time 14 day measurement period, the N_2O emissions decreased from 1.89 to 0.46 ng m^{-2} s^{-1} and 2.16 to 0.55 ng m^{-2} s^{-1} for the NH_4NO_3 and $NaNO_3$ treated columns, respectively. N_2O emissions for the other treatments were low or zero. No emission was observed for the NH_4Cl treatment columns and was only observed on the first day of measurement for the control columns. For the NaCl treatment N_2O emission ranged between 0.27 to 0.71 ng m^{-2} s^{-1}. Soil pH was not significantly different between treatments averaging pH 3.3.

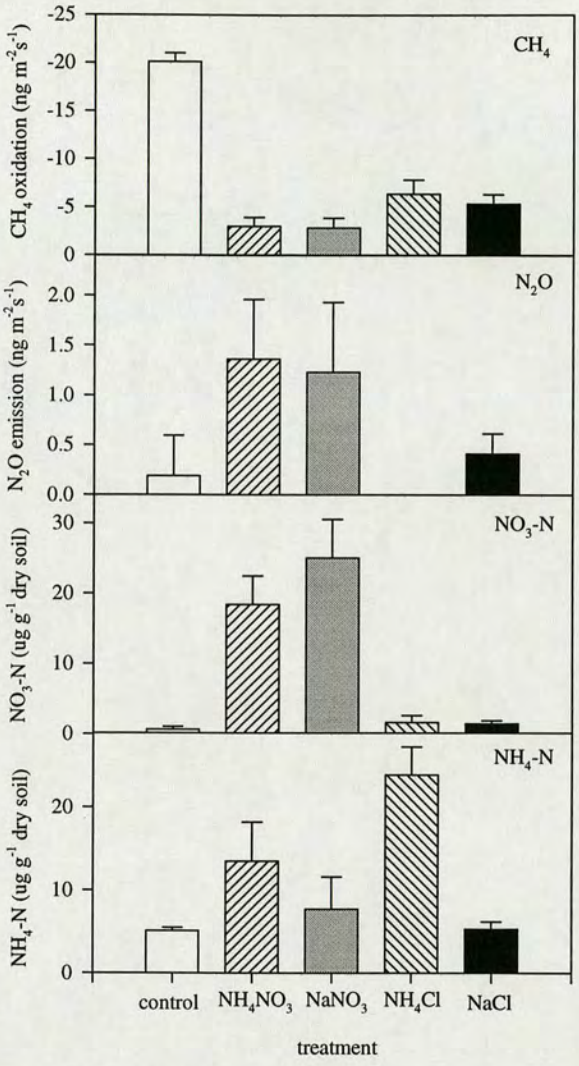

Figure 4.7. Mean CH_4 oxidation rates, N_2O emission rates and soil available N concentrations from N treated soil columns over the 14 day measurement period. Error bars are the standard deviation from the mean.

4.5 Discussion

Methane oxidation rates showed significant seasonal variability and soil column manipulation experiments under controlled conditions confirmed the field observations. N deposition at the hill summit affected the magnitude of the sink strength. The responses of CH_4 oxidation rates to season and N input are discussed in turn and the results of manipulation experiments are discussed in conjunction with field observations.

4.5.1 Seasonal response of CH_4 oxidation

Large seasonal variations in CH_4 oxidation rates, with summer maxima, were observed over the two measurement years. The strong correlations with soil temperature suggested that the dominant control on oxidation rates was the temperature dependence of the methanotrophs. Soil water content and rainfall had a much smaller effect. The soil water content and temperature manipulation experiment confirmed the temperature response observed in the field and showed that temperature exerted the dominant influence on CH_4 oxidation rates over the soil water content range commonly observed in the field. This observation contradicts several studies where soil water content has generally been found to exert the dominant control on temporal changes in CH_4 oxidation rates (Lessard *et al.*, 1994, Keller & Reiners, 1994, Castro *et al.*, 1995) and where temperature has been found only to have a weak influence on CH_4 oxidation rates (King & Adamsen, 1992, Dorr *et al.*, 1993, Lessard *et al.*, 1994). Q_{10} and E_a values at the low altitude forest and moorland sites were larger than those reported in similar studies, which generally range between 1 and 2 for Q_{10} (Crill, 1991, King & Adamsen, 1992) and between 20 and 80 kJ mol^{-1} (Dunfield *et al.*, 1993, Crill *et al.*, 1994). The weak temperature dependence commonly observed is unusual for a biological process, and has been attributed to a limitation of the rate of diffusion of substrate to microorganisms by physical factors, such as soil water content and texture (Dorr *et al.*, 1993, Castro *et al.*, 1995). The large temperature dependence observed in this study may have been due to the high gas diffusivity within the peat. The soil column water content and temperature manipulation showed that CH_4 oxidation rates did not significantly differ in the water content range 80 to 180 % dry weight, suggesting that, over this range, the peat was sufficiently porous to allow substantial fluctuations in air filled pore space without restricting the gas transport of CH_4 and O_2 to CH_4 oxidisers. The absence of any diffusion limitation would allow the CH_4 oxidisers to respond readily to changes in soil temperature, providing that there were no other rate-limiting factors. Dunfield *et al.* (1995) also observed a diffusion limitation only at high soil water contents (>130 % dry weight) in a humisol, and concluded that below this soil water content diffusion was fast in relation to the microbial oxidation rate. Soil water content manipulations generally show a plateau region (Nesbit & Breitenbeck, 1992, Dunfield *et al.*, 1995, Castro *et al.*, 1995) in which the flux is insensitive to changes in soil water content, due to the absence of a diffusion limitation. As soil water content increases and restricts diffusion, oxidation rates decrease (Nesbit & Breitenbeck, 1992, Dunfield *et al.*, 1995). The size of the plateau region of the curve is regulated by soil properties such as soil texture and bulk density. Within the plateau region CH_4 oxidation

rates will be solely determined by factors other than diffusional controls, such as soil temperature (this study), or soil fertility (Castro *et al.*, 1995). The relatively small CH_4 oxidation rates observed in the high soil water content treatment is probably due to a restriction of the diffusion of substrate to methanotrophs, or CH_4 production may be mediating the net oxidation rate (Yavitt *et al.*, 1990). CH_4 oxidation rates were weakly correlated with the previous 24 hours rainfall. CH_4 oxidation rates can be periodically mediated in the short term by rainfall acting as a diffusion barrier, as demonstrated by Castro *et al.* (1994).

Seasonal variations in CH_4 oxidation rates therefore appear to be controlled by a combination of factors including soil gas diffusivity, soil water content and soil temperature. The interaction and relative importance of each factor appears to depend on soil type and CH_4 oxidation potential. In this study soil temperature exerted the dominant effect as a result of high soil gas diffusivity.

A temperature optimum of ~ 20 °C was observed from the temperature manipulation experiment. Temperature optima of ~25 °C in peat slurries (Dunfield *et al.*, 1993), 31 °C in a landfill cover soil (Whalen & Reeburgh, 1990) and between 20 and 30 °C for a swamp and forest soil, respectively (Nesbit & Breitenbeck, 1992) have been observed. The slightly lower temperature optimum found in this study may have been due to CH_4 production in anaerobic microsites. CH_4 production is strongly affected by soil temperature, therefore at high soil temperatures CH_4 production could mediate net CH_4 oxidation rates.

The dependence of CH_4 oxidation rates on soil temperature may lead to an increase in the CH_4 soil sink strength in these soils in response to increasing surface temperatures, as predicted by climate change models (IPCC, 1994). The effect may be enhanced if soils become generally drier; however, if precipitation increases sufficiently to more than offset the increased potential evaporation, then the net CH_4 sink strength may decline as gaseous diffusion in soil becomes increasingly restricted by the addition of soil water.

Methane oxidation rates were at the lower end of the range reported for other studies. In temperate forest soils in the NE United States, oxidation rates averaging -33.0 and -18.0 ng m^{-2} s^{-1} were observed by Steudler *et al.* (1989) and Crill (1991), respectively. Dorr *et al.* (1993) observed rates ranging from -2.8 to -30.5 ng m^{-2} s^{-1} in forest soils of SW Germany. Whalen & Reeburgh (1990) found oxidation rates averaging -31.2 ng m^{-2} s^{-1} from Alaskan tundra, and in montane meadow soils in Colorado Torn *et al.* (1996) found rates averaging -13.9 ng m^{-2} s^{-1}. The mean fluxes found in this study ranged between -2.7 and -7.9 ng $m^{-2}s^{-1}$ and were close to those observed by Castro *et al.* (1993), in spruce fir forests of the

NE United States which ranged between -5.5 and -13.9 ng m^{-2} s^{-1}. The small rates may be due to a combination of factors including the low mean soil temperature (~7 °C) and the low pH of the site (Table 4.2 and 4.3), resulting in a generally low microbial activity.

4.5.2 The effect of N deposition on CH_4 oxidation rates

Rates of CH_4 oxidation were significantly smaller at the high altitude forest site than at the low altitude forest site for approximately half the year, during the summer months, and throughout the year at the moorland site. The most apparent difference between the three sites was in their atmospheric deposition loading of N and soil water content (Table 4.1). The hill summit was frequently in cloud and has much larger inputs of deposited N species. Because the trees act as interceptors for deposited species, which are then washed off by rainfall into the soil, deposition rates within the canopy may be up to 90% higher (Fowler *et al.*, 1989). This process resulted in the available NH_4^+-N soil concentrations observed at the top forest site being ~60% larger than at the adjacent moorland site, and in a decrease in CH_4 oxidation rates by between 46 and 61% at the high altitude forest relative to the low altitude forest and moorland sites, respectively. N_2O emission rates increased by 500% compared to the low altitude forest, which received 3 times less atmospheric N (U. Skiba pers. comm.). This response to increased N was repeated in a laboratory study where inhibition of CH_4 oxidation rates was greater than 70 % as a result of various N inputs. However, one could also argue that the field observations of inhibition of CH_4 oxidation were a result of the larger soil water contents at the high altitude forest site. The differences in soil water contents are likely to enhance the observed differences in flux between the sites, but are unlikely to be solely responsible for this observation. Firstly, laboratory experiments showed that CH_4 oxidation rates were very robust against changes in soil water content between 80 and 180% dry weight. This was also the range of soil water contents observed in the field. Secondly, in spite of the lower rainfall in the week preceding the flux measurements in 1995 compared to 1994, CH_4 oxidation rates were the same for both periods. Thirdly, the largest CH_4 oxidation rates were observed at the moor site, which was even wetter than the low forest soil. Obviously, parameters other than soil water content were controlling the flux.

Inhibition of CH_4 oxidation rates by various forms of N has been reported in many soils in the field and laboratory. Steudler *et al.* (1989) reported up to a 33% reduction in oxidation rates following fertilisation with 120 kg N ha^{-1} y^{-1} as NH_4NO_3 in temperate forest soils, Sitaula *et al.* (1995) observed a reduction in oxidation rates of 15% and 38%

following application of 30 and 90 kg N ha^{-1} y^{-1} of NH_4NO_3 to lysimeter soil from a Scots pine forest, and Adamsen & King (1993) observed a strong (up to 95%) inhibition, following fertilisation with 20 kg N ha^{-1}, and found a greater effect in subarctic than temperate forest soils. However, results from many N addition studies show conflicting evidence about the magnitude, duration and mechanism of the inhibition. For example, no inhibition was detected following fertilisation with 100 kg N ha^{-1} either as urea or $NaNO_3$ in a humisol (Dunfield & Knowles, 1995). It has been suggested that NH_3 can act as a comepetitive inhibitor of CH_4 oxidation, due to the similarity of the mono-oxygenase enzyme systems catalysing the initial stage of CH_4 and NH_4 oxidation (Bedard & Knowles 1989). However, the inhibition has been shown to last for long periods after added NH_4 has returned to background levels (Mosier *et al.*, 1991, Nesbit & Breitenbeck, 1992, Willison *et al.* 1995). The positive correlation observed in this study between the soil NH_4-N and CH_4 oxidation rates was probably a result of seasonal variations in nitrification rates; however, negative correlations have also been observed (Dobbie *et al.*, 1996). Mosier *et al.* (1991) has speculated that N turnover rates rather than mineral N content may be affecting CH_4 oxidation rates and negative correlations between nitrification rates and CH_4 oxidation rates have been observed (Sitaula *et al.*, 1993). However, King & Schnell (1994) have speculated that the persistence of the inhibition may be related to the limited capacity of methanotrophs to grow on atmospheric CH_4 concentrations. The inputs of atmospheric N, soil available NH_4-N and CH_4 fluxes are summarised schematically in Figure 4. 8.

The N addition experiment reported here resulted in a strong (> 70%) inhibition of CH_4 oxidation rates in the N-treated cores, relative to the control cores. The NO_3-N treated cores resulted in the largest inhibition of oxidation rates, coincident with the largest soil available NO_3-N concentrations and N_2O emissions, indicating that some denitrification was occurring. The evidence for inhibition by NO_3-N is conflicting; generally, studies have found a greater degree of inhibition by NH_4-N than NO_3-N (Adamsen & King, 1993, Dunfield & Knowles, 1995), and both Nesbit & Breitenbeck (1992) and Willison *et al.* (1995) found no inhibitory effect of NO_3-N. Crill *et al.* (1994) provided evidence that the inhibition by KNO_3 observed could be a result of remineralisation of N as NH_4, or the liberation of NH_4^+ from exchange sites by the added K^+. In this study soil analysis indicated that either remineralisation and liberation, or exchange had only a very small effect.

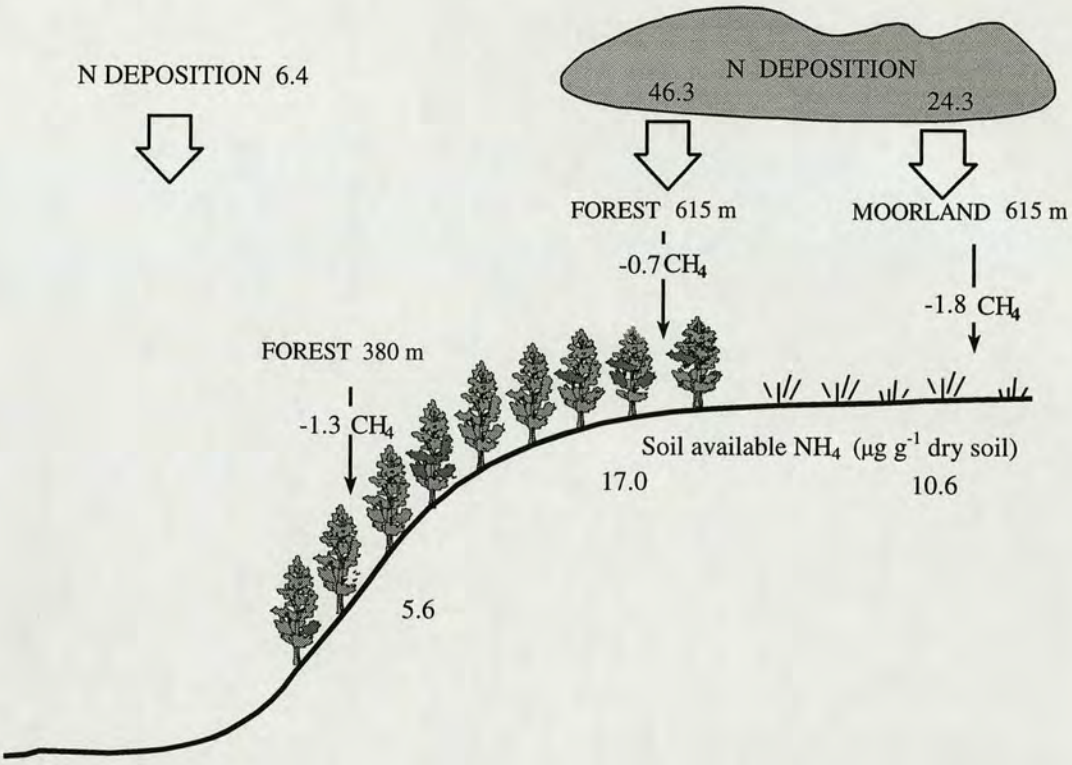

Figure 4.8. Schematic representation of annual fluxes of CH_4 (kg ha^{-1} y^{-1}), soil available NO_3-N and NH_4-N, and N deposition rates at Dunslair. The hill summit is frequently in cloud and has much larger inputs of deposited N species. The total annual deposition of N at the site increases from 6.4 kg ha^{-1} at 275 m to 24.3 kg ha^{-1} at 615 m, the summit (Crossley *et al.*, 1992). Because the trees act as interceptors for deposited species, which are then washed off by rainfall into the soil, within canopy deposition rates were up to 90% higher (Fowler *et al.*, 1989). This resulted in the soil available NH_4-N concentrations observed at the top forest site being ~60% larger than at the adjacent moorland site

The NH_4Cl treated cores inhibited oxidation rates significantly less than the treatments containing NO_3-N. No N_2O was emitted from the NH_4Cl treatment and soil available NH_4-N concentrations decreased as NO_3-N concentrations increased over the measurement period as a result of nitrification. Available NH_4-N concentrations measured across the range of treatments had no effect on the degree of inhibition observed (Fig 4.7). NH_4 concentrations have been shown to have no effect on the degree of inhibition in several studies (Sitaula *et al.*, 1993, Crill *et al.*, 1994). However, dose-related effects of added NH_4-N have been reported elsewhere (Adamsen & King, 1993, Dunfield & Knowles, 1995, Boeckx and Van Cleemput, 1996).

The observation that an equivalent amount of NaCl resulted in inhibition of a similar magnitude to that found for NH_4Cl treatment questions the competitive inhibition hypothesis. Soil available NH_4-N concentrations did not differ significantly from the control soils, so it is difficult to argue that the Na^+ is simply releasing NH_4^+ from exchange sites.

However, soil available NO_3-N concentrations did increase over the measurement period relative to the control soils (Table 4.3). Therefore some mineralisation and nitrification was taking place, also indicated by the N_2O emission. However, the concentrations of soil available N measured were an order of magnitude smaller than those observed in the N treated cores. Salts have been observed to inhibit CH_4 oxidation rates in other studies (Adamsen & King, 1993, Dunfield & Knowles, 1995) but the mechanism responsible is unknown. Salts have been shown to have an adverse affect on other microbial processes such as nitrification (Martikainen, 1985) which was attributed to a pH effect. Nesbit & Breitenbeck (1992) suggested that inhibitory effects may be due to the sensitivity of methanotrophs to an increase in osmotic potential and Knightley *et al.*, (1995) suggested that a change in ionic strength as opposed to competitive effects of NH_4 may be responsible for the inhibition.

The available N concentrations in the control cores remained stable over the measurement period compared to the N concentration changes occurring in the other treatments. (Table 4.3) This supports the suggestion of Mosier *et al.* (1991) that N turnover, rather than NH_4-N concentrations, were responsible for the inhibition. However, CH_4 oxidisers in this soil would necessarily have been extremely sensitive to any changes in N concentrations, as indicated by the very small N concentration changes observed in the NaCl-treated core.

King & Schnell (1994) suggested that nitrite formation from methanotrophic NH_4^+ oxidation resulted in a non-competitive inhibition of CH_4 oxidation. There is some evidence for nitrification in this soil, as indicated by the decreasing available NH_4-N concentrations observed most clearly in the NH_4Cl-treated core. Nitrification by nitrifiers is unlikely in this soil due to the low pH and temperature (Saad & Conrad, 1992, Laanbroek & Woldendorp, 1995). If the addition of NH_4-N resulted in nitrification by methanotrophs then it is likely that the products hydroxylamine and nitrite would have had toxic effects. The N-treated cores containing NO_3-N had the strongest inhibitory effect. Another path for NO_2^- may be through denitrification of added NO_3, as indicated by the N_2O emission. NO_2^- has been shown to be more effective at inhibiting CH_4 oxidation than NH_4 (Schnell & King, 1994).

4.6 Summary

Methane oxidation rates showed significant seasonal variations in response to soil temperature. Soil column manipulations under controlled conditions showed that, over the range observed in the field, soil water content had no significant effect on CH_4 oxidation

rates. This was attributed to the large gas diffusivity of the peat. Small CH_4 oxidation rates in the forested site at the hill summit were coincident with a large input of N from atmospheric pollution and consequently large available NH_4-N concentrations in the soil. An N manipulation investigation on soil columns under controlled conditions showed that CH_4 oxidation rates were inhibited by >70% by additions of NH_4NO_3, $NaNO_3$, NH_4Cl and NaCl. The mechanism of the inhibition was most likely a combination of several processes; changes in N turnover resulting in NO_2^- production, sensitivity of microrganisms to changes in osmotic potential, and competitive inhibition between NH_3 and CH_4. The low CH_4 oxidation rates found at the high altitude forest site may therefore be a result of the deposition of pollutant species other than N, and the enhanced input of all ions as well as NH_4^+ and NO_3^- may have inhibited CH_4 oxidation rates. Atmospheric pollution input may therefore have reduced the soil sink capacity for CH_4 by approximately 50% at polluted high elevation sites.

Chapter Five

Seasonal variation in CH_4 oxidation rates in two mineral forest soils

5.1 Abstract

Rates of CH_4 oxidation in two mineral forest soils were measured over a period of ~1 year in order to investigate temporal and spatial variability. CH_4 oxidation rates ranged from -0.2 to -18.8 ng m^{-2} s^{-1}. Seasonal variability was determined by soil water content and temperature with significant correlations ($p<0.05$) being observed with CH_4 oxidation rates. Soil water content appeared to have the dominant influence on oxidation rates and a non-linear sigmoidal response to water content was observed at a grassland site. A significant negative correlation ($p<0.05$) was observed between rates of CH_4 oxidation and soil bulk density, indicating that the gaseous diffusion of CH_4 to methanotrophs was determining spatial variability. N fertilisation of a grass plot (265 kg N ha^{-1}) resulted in an average decrease in CH_4 oxidation rates of 23%, and low CH_4 oxidation rates were associated with large soil NO_3-N concentrations in a N-fixing alder plot. However, NH_3 deposition (<4 kg N ha^{-1}) did not result in a significant inhibition of CH_4 oxidation rates.

5.2 Introduction

Mineral forest soils are one of the most extensively studied environments with regards the oxidation of CH_4 (Crill, 1991, Castro *et al.*, 1995, Sitaula *et al.*, 1995), and are an important component of the global soil sink strength. Some of the largest rates of CH_4 oxidation have been observed in temperate forest soils (Steudler *et al.*, 1989, Crill, 1991) and Ojima *et al.* (1993) calculated that without a temperate soil sink of 20 $Tg\,y^{-1}$ atmospheric CH_4 concentrations would be increasing at 1.5 times the present rate. Seasonal variability in rates of CH_4 oxidation have been found to be affected to varying degrees by soil water content (Castro *et al.*, 1994, Lessard *et al.*, 1994), and soil temperature (Crill, 1991, Castro *et al.*, 1995). In general, soil water content has been observed to be the major influence affecting rates of CH_4 oxidation, attributed to the diffusion of CH_4 to methanotrophs being the rate-controlling factor (Dorr *et al.*, 1993, Lessard *et al.*, 1994). However, soil temperature has also been observed to be important in some soils (eg. MacDonald *et al.*, 1997/Chapter 4). Spatial variability may be influenced by a wide range of parameters including soil water content (Czeipel *et al.*, 1995), soil texture (Dorr *et al.*, 1993, Keller & Reiners, 1994), soil bulk density (MacDonald *et al.*, 1996/Chapter 3), soil organic matter (Czeipel *et al.*, 1995), N fertilisation (Steudler *et al.*, 1989, Situala *et al.*, 1995), site fertility (Castro *et al.*, 1995), pH (Hŋtsch *et al.*, 1994) and land use (Lessard *et al.*, 1994, Dobbie *et al.*, 1996).

In this study, CH_4 oxidation rates were measured from two mineral forest soils over a period of between 9 and 11 months, in order to investigate the influence of seasonal variations in soil water content and temperature on CH_4 oxidation rates. Plots were also selected to investigate the effect of N input, by measuring CH_4 oxidation rates from an N fixing alder plot, and at a forest site upwind and downwind of an NH_3 source. A field manipulation experiment was also carried out to investigate the effects of N fertilisation to grassland.

5.3 Materials and Methods

5.3.1 Site Description

Glencorse (NT 241631) - an experimental field site near Edinburgh, S.E. Scotland (186 m asl) which was converted from arable/pasture to forest in 1984. Sites were established in plantations of Red alder, common birch, Sitka spruce and rye-grass. The soil was a brown forest soil of the Macmerry/ Winton series (Brown & Shipley, 1982), textural class - clay loam.

Devilla (ND 065453) - a 40 year old lowland Scots Pine plantation near Kincardine, Fife, E.

Scotland (65m asl), planted in 1952 on a brown forest soil, with gleying, of the Rowanhill association (Brown & Shipley, 1982). The soil was a sandy clay loam overlain by an organic layer of varying thickness. The forest was a site for a $(NH_4)_2SO_4$ co-deposition experiment (see Cape *et al.*, 1995) and sites were established on a transect away from a NH_3 source. NH_3 gas was released above the canopy in proportion to wind speed and direction. The control site was established 15-20 m upwind of the NH_3 source. Two sites, 'near NH_3' and 'far NH_3' were established 10-15 m and 25-30 m downwind of the NH_3 source.

5.3.2 Field measurements of CH_4 oxidation rates and soil parameters

Field measurements were made using the static chamber method as described in Chapter 2. Soil samples were analysed for water content, temperature, available NO_3-N & NH_4-N, bulk density and pH as described in Chapter 2. Measurements of soil diffusivity were made by Bruce Ball (SAC) using a freon tracer technique (Ball *et al.*, 1994). At Glencorse three chambers were installed randomly in each plot and CH_4 oxidation rates were measured 16 times between April 1994 and February 1995. At the grass plot, which acted as a control for the N fertilisation experiment, oxidation rates were measured 27 times throughout the measurement period. At Devilla three chambers were initially installed at each site. Following the first four sampling days, control chambers were moved in an attempt to find soil conditions more similar to the near and far NH_3 sites. On 21 Oct. 1994 the number of chambers at each site was increased to five.

5.3.3 Nitrogen fertilisation investigation, Glencorse

A 30 m^2 grass plot was fertilised with 165 and 100 kg ha^{-1} NH_4NO_3, on two occasions, in April and July, respectively. Three chambers were installed and measurements were made 27 times between 24 April 1994, and 19 Jan 1995. The grass plot described above (5.3.1) was measured in parallel as a control.

5.3.4 Statistical analysis

Methane oxidation rates from each chamber were meaned for each measurement day. Relationships between fluxes and environmental parameters were investigated using the Pearson product moment correlation and simple linear regression. Significant differences between sites were determined using analysis of variance (ANOVA).

5.4 Results

5.4.1 Field measurements at Glencorse

Net rates of CH_4 oxidation were observed throughout the measurement period at all four sites and ranged from -0.2 to -18.8 ng m^{-2} s^{-1} (Figure 5.1). The largest CH_4 oxidation rates were found in the sitka plot ($p<0.05$). Oxidation rates in the grass and birch plots were not significantly different throughout the year and the smallest rates of CH_4 oxidation were observed in the alder plot ($p<0.05$), (Table 5.1). Rates of CH_4 oxidation showed a seasonal dependence and significant linear correlations were observed with soil water content and temperature at all sites ($p<0.05$). Soil water content and temperature were also significantly correlated ($p<0.05$) at all sites, and were not significantly different throughout the year between the four sites ($p<0.05$). At the grass plot, where a greater number of measurements were made (n=27) than at the other sites (n=16), a sigmoidal relationship (r^2 = 0.787, $p<0.001$) was observed and a four parameter logistic function was fitted to the curve. The relationship between soil water content and CH_4 oxidation rate at the grass control site is shown in Figure 5.2. At soil water contents less than 25 % (dry weight) no dependence of CH_4 oxidation rates on soil temperature or soil water content was observed, despite a variation in soil temperature between 12 and 17 °C and a 10% variation in soil water content. At soil water contents greater than 25 % (dry weight) oxidation rates decreased as soil water content increased, and at soil water contents greater than about 35% the rate of change appeared to decrease to a plateau. A significant relationship between oxidation rates and soil temperature was also observed at the grass site. An exponential curve gave the best fit through the data points ($p<0.001$, r^2= 0.602) (not shown).

Average rates of CH_4 oxidation at all sites and the soil parameters measured are shown in Table 5.1. Soil available NH_4-N and NO_3-N concentrations showed a significant positive correlation with CH_4 oxidation rates at the grass site ($p<0.01$). Soil available NO_3-N concentrations were significantly larger and the soil pH was more acidic in the alder site ($p<0.05$) compared to the other sites. Soil diffusivity and soil bulk density followed a similar trend between sites with the highest diffusivity and lowest bulk density observed at the sitka site, and the lowest diffusivity and highest soil bulk density at the alder site.

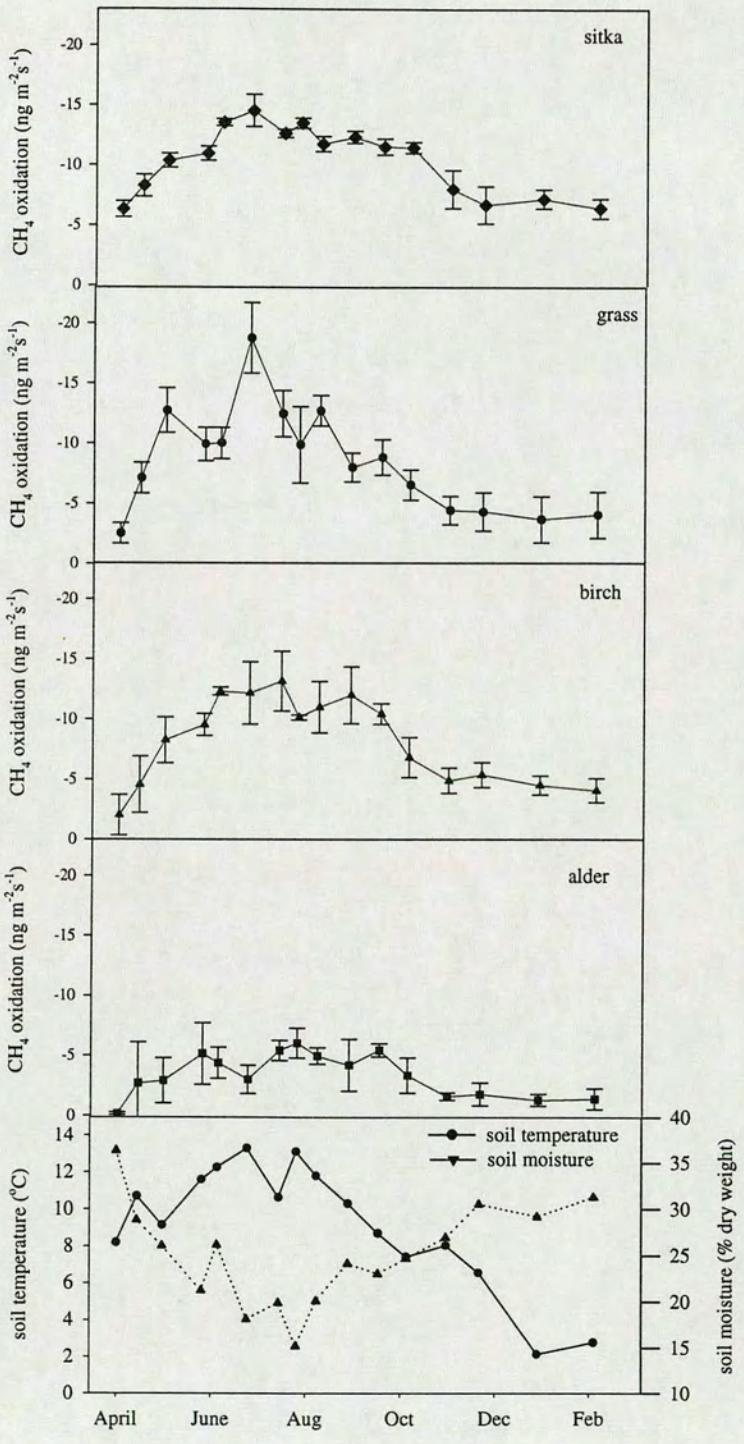

Figure 5.1. The seasonal variation in CH_4 oxidation rates in the sitka, grass, birch and alder sites at Glencorse, and the mean soil water content and soil temperature from all the sites throughout the measurement period. Error bars represent the standard deviation from the mean.

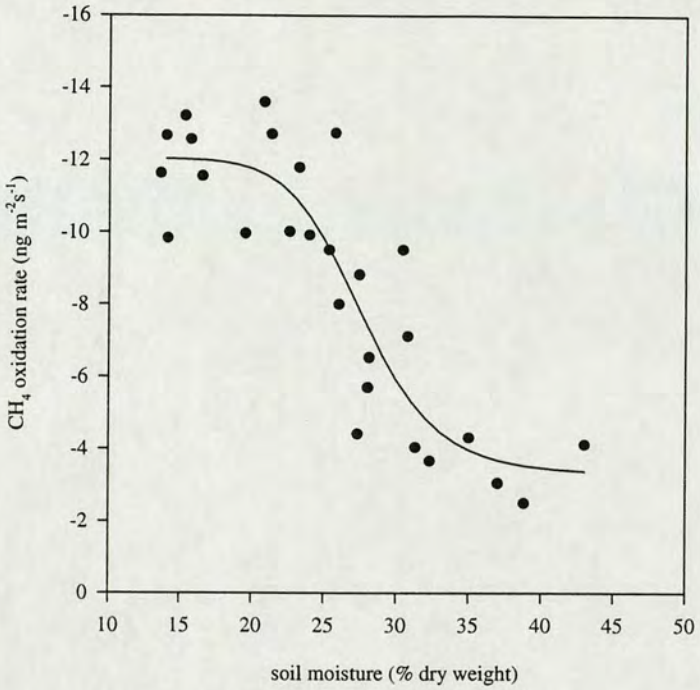

Figure 5.2. The response of CH_4 oxidation rates in the grass plot, Glencorse to seasonal variations in soil water content. A four-parameter logistic function was fitted to the data such that:
CH_4 oxidation rate (ng m^{-2} s^{-1}) = 8.7/(1+(%H_2O/27.7)exp10.6)+3.3 (r^2 = 0.787, $p<0.001$).

5.4.2 Field measurements at Devilla

Methane oxidation rates ranged from -1.9 to -14.2 ng m^{-2} s^{-1} and significant differences were observed between the three sites ($p<0.05$), with the largest rate of CH_4 oxidation being found in the far NH_3 site and the smallest rate in the near NH_3 site, with the control site showing rates of oxidation between the two. Annual variation in CH_4 oxidation rates, soil water content and temperature are shown in Figure 5.3. A significant correlation with soil water content was only observed at the far NH_3 site and with soil temperature at the near NH_3 site ($p<0.05$). No significant differences in soil water content, temperature or soil available NO_3-N and NH_4-N were found between the three sites over the measurement period ($p>0.05$).

5.4.3 Field measurements at Glencorse and Devilla

Mean CH_4 oxidation rates, from each site, over the same measurement period were significantly correlated with soil bulk density (r^2=0.651, $p<0.05$) (Figure 5.4). Bulk density measurements from the sites at Glencorse compared well with the soil gas diffusivity measurements, and may therefore be used as an indicator of soil gas diffusivity.

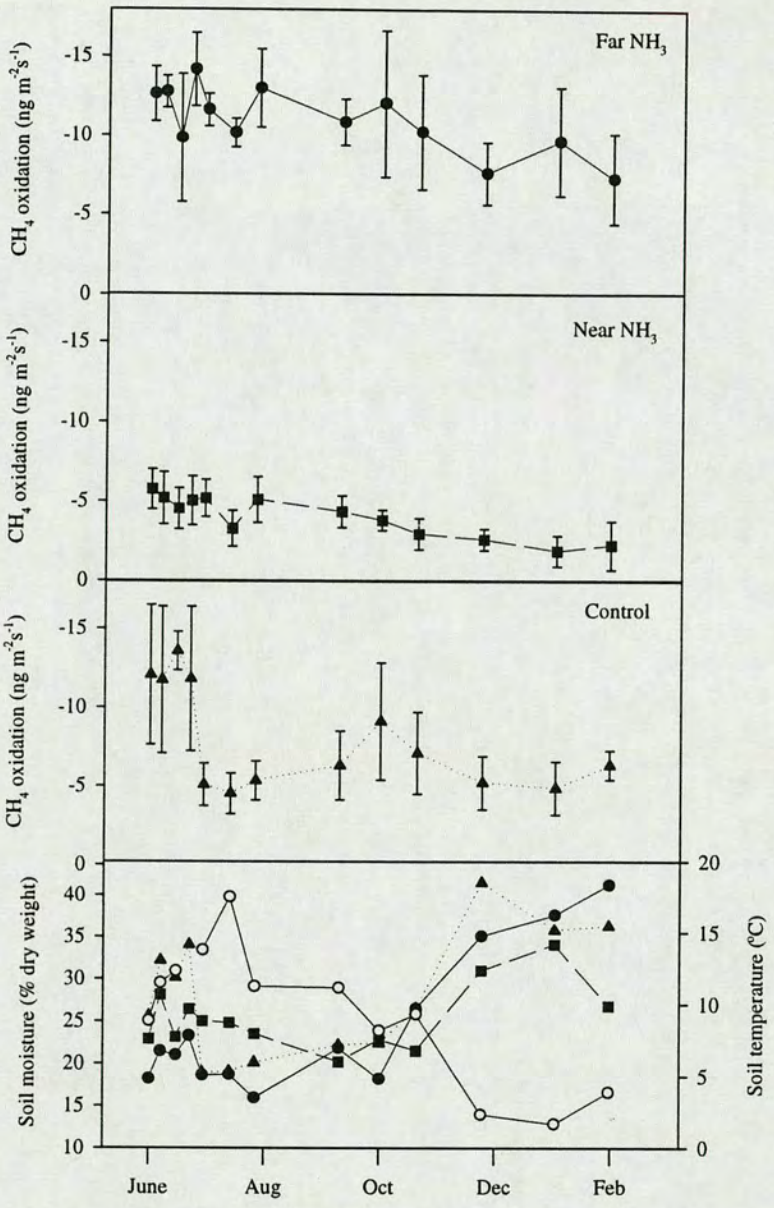

Figure 5.3. Seasonal variation in CH_4 oxidation rates from the (●) far NH_3, (■) near NH_3 and (▲) control sites at Devilla, and soil water content (●■▲) and mean soil temperature (○) for all three sites throughout the measurement period. Error bars represent the standard deviation from the mean.

5.4.4 Nitrogen fertilisation investigation, Glencorse.

Methane oxidation rates from the fertilised grass plot were significantly ($p<0.05$) lower than from the control plot, with an average reduction of 23%, over the measurement period (Figure 5.5). Soil available NH_4-N and NO_3-N concentrations were higher in the fertilised than in the control plot (Table 5.2). Soil water content and temperature were not significantly different between the plots ($p>0.05$).

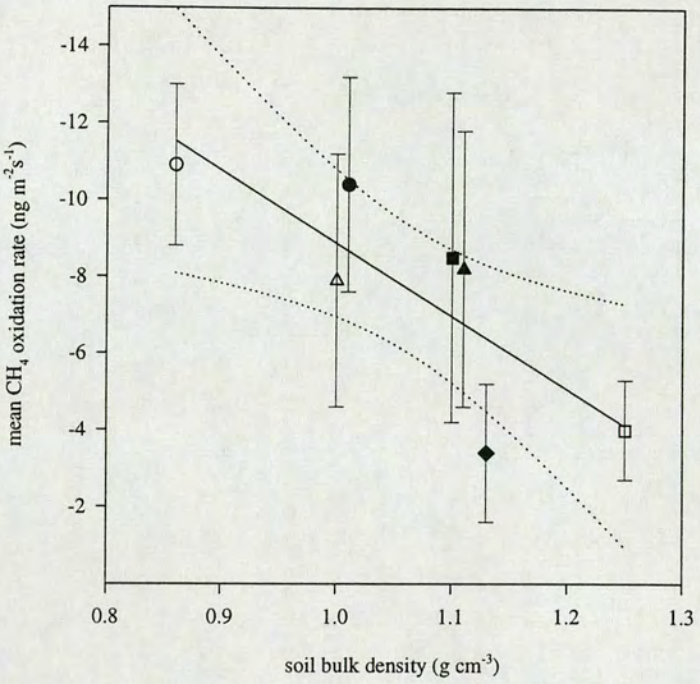

Figure 5.4. The relationship between mean CH_4 oxidation rates and soil bulk density at each plot in both forest sites studied: ◆ alder, ▲ birch, ● sitka, ■ grass, □ near NH_3, ○ far NH_3 and ▽ control. The regression equation between soil bulk density and CH_4 oxidation rates was: CH_4 oxidation rate = 18.6*soil bulk density+27.7 (r^2=0.609, $p<0.05$). The oxidation rates from both sites cover the period June 94 to Feb. 95. The error bars at each site are the standard deviation from the mean and indicate the magnitude of the seasonal variability. The dotted lines show the 95% confidence limits of the regression.

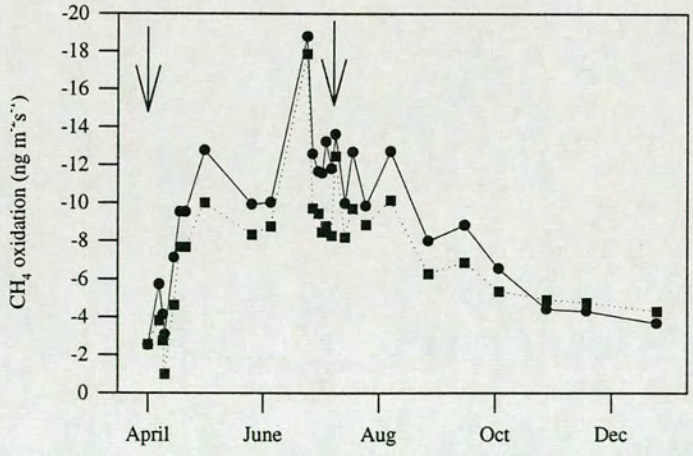

Figure 5.5. CH_4 oxidation rates from the fertilised (■) and control (●) grass plots at Glencorse. The arrows represent fertiliser application (100 kg N ha^{-1} NH_4NO_3 in April and 165 kg N ha^{-1} NH_4NO_3 in July 1994).

Table 5.1. Rates of CH_4 oxidation and soil parameters from Glencorse and Devilla.

Site	Mean CH_4 oxidation rate (ng m^{-2} s^{-1})	Mean soil water content (% dry wt.)	Mean soil temp. (°C)	Mean NO_3-N (:g g^{-1} dry soil)	Mean NH_4-N (:g g^{-1} dry soil)	pH	organic matter content (%)	soil bulk density (g cm^{-3})	soil diffusivity (mm^{-2} s^{-1})
Glencorse									
Sitka	-10.4 (2.8)[a]	23.4	8.3	1.2	3.5	4.4	9.0	1.01	0.613
Grass	-8.7 (4.3)	26.4	10.4	1.4	5.5	4.9	9.5	1.10	0.544
Birch	-8.2 (3.6)	23.7	9.1	1.7	5.0	4.2	7.1	1.11	0.500
Alder	-3.3 (1.80	26.8	8.9	6.0	3.1	3.8	6.0	1.13	0.115
Devilla									
Far NH_3	-10.9 (2.1)	24.4	9.2	1.57	6.58	3.4	8.8	0.86	nd
Near NH_3	-4.0 (1.3)	25.4	9.3	1.42	4.70	3.2	11.0	1.25	nd
Control	-7.9 (3.3)	28.1	9.4	2.70	4.60	3.4	11.0	1.0	nd

[a] = mean of each chamber measurement ()= standard deviation nd = not determined

Table 5.2. Mean CH_4 oxidation rates, soil available NO_3-N and NH_4-N, and pH from the N fertilisation investigation.

	Mean CH_4 oxidation rate (ng m^{-2} s^{-1})	Mean soil NO_3-N (:g g^{-1} dry soil)	Mean soil NH_4-N (:g g^{-1} dry soil)	pH
Control grass	-9.3 (4.2)[a]	2.9 (3.7)	5.1 (3.2)	4.9
Fertilised grass	-7.2 (3.6)	32.0 (47.3)	37.1 (49.9)	4.8

[a]mean () standard deviation
n = 81 for CH_4 oxidation rates

5.5 Discussion

5.5.1 Seasonal variability at Glencorse and Devilla

Rates of CH_4 oxidation at both sites showed a response to seasonal changes in soil water content and temperature. Seasonal variability in CH_4 oxidation rates has been observed in many studies, however, the magnitude of the seasonal response varies between sites. Lessard *et al.* (1994) observed a stronger response to soil water content than to soil temperature, and Crill (1991) observed a response to soil temperature only in spring suggesting this was a result of a change from biological to diffusional control. Dorr *et al.* (1993) found a seasonal response, attributed to soil water content, in a loam soil but not in a sandy or a clay soil. The relative response of CH_4 oxidisers to soil water content and temperature is of interest, particularly when trying to predict the effects of changing climate, or scale up to annual fluxes. The influence of soil water content may be mediated through the restriction of gaseous diffusion of CH_4 to methanotrophs (Dorr *et al.*, 1993), or simultaneous production of CH_4, mediating the net oxidation rate (Yavitt *et al.*, 1990). Soil temperature acts directly on the microbial community, however, it has been widely observed that CH_4 oxidation rates are less responsive to temperature than are other microbial processes (King & Adamsen, 1992, Dorr *et al.*, 1993, Lessard *et al.*, 1994, Dobbie & Smith, 1996), as a result of the diffusional control on CH_4 oxidation rates (Dorr *et al.*, 1993). However, MacDonald *et al.*, (1997)/Chapter 4 observed a strong temperature response in a peaty podzol and attributed this observation to the high gas diffusivity of the soil. Therefore temperature can be important in some soils or at certain times of year. At Glencorse the greatest response of CH_4 oxidation rates to seasonal variations in both soil water content and temperature occured in the spring as the soils dried out and temperature increased. During the summer period oxidation rates reached their maximum rate and despite further variations in soil

water content and temperature remained stable, probably as a result of a diffusional limitation exerted by the texture of the soil. As soil water content increased and temperature decreased in the autumn CH_4 oxidation rates fell. However, between December and February CH_4 oxidation rates remained stable despite a large decrease in soil temperature to less than 3 °C, indicating that at this time of year soil temperature was not an important factor. At Devilla the responses to soil water content and temperature were not as significant as those observed at Glencorse, in part due to the higher spatial variability at the Devilla site and also due to the shorter measurement period. (CH_4 oxidation rates were not measured in the spring where the largest responses to soil water content and temperature changes were observed at Glencorse).

In the grass plot, the regression between soil water content and CH_4 oxidation rates showed a sigmoidal response (Figure 5.2). In the plateau region, at soil water contents less than 25% (dry weight), it seems likely that soil diffusivity, as determined by soil texture and organic matter content, was controlling CH_4 oxidation rates by determining the rate of gaseous diffusion of CH_4 to methanotrophs. Therefore changes in temperature and water content had no further effect on the microbial community. A sigmoidal fit was used to describe the data observed in the grass plot. However, microorganisms are known to become physiologically stressed at low water potentials (Adamsen & King, 1993), and over a wider range of soil water contents the CH_4 oxidation rate would decrease as the microorganisms became inhibited by the low soil water content. As the soil water content increased and restricted the diffusion of CH_4 to methanotrophs, CH_4 oxidation rates decreased, and would presumably have decreased to zero, until the balance of CH_4 production versus CH_4 oxidation resulted in a net CH_4 emission to the atmosphere. Non-linear responses of CH_4 oxidation rates to variations in soil water content have been observed elsewhere (Czeipel *et al.*, 1995, Dunfield *et al.*, 1995), although frequently, the range of soil water contents studied is such that only the linear portion of the relationship can be observed (eg. Lessard *et al.*, 1994).

When all the sites were combined the responses to both soil water content (Figure 5.6) and temperature (Figure 5.7) fell into two groups, apparently dependent on the soil diffusivity/ bulk density status of each soil; low diffusivity (alder, Near NH_4) and high diffusivity (sitka, birch, grass, Control, Far NH_4). The response of CH_4 oxidation rates to soil water content was linear. Both groups of data showed a significant response, with the high diffusivity sites ($p<0.001$, $r^2 = 0.527$) at -0.3 ng m^{-2} % (dry $weight^{-1}$) and the response of the low diffusivity sites at -0.2 ng m^{-2} % (dry $weight^{-1}$) ($p<0.05$, $r^2 = 0.456$).

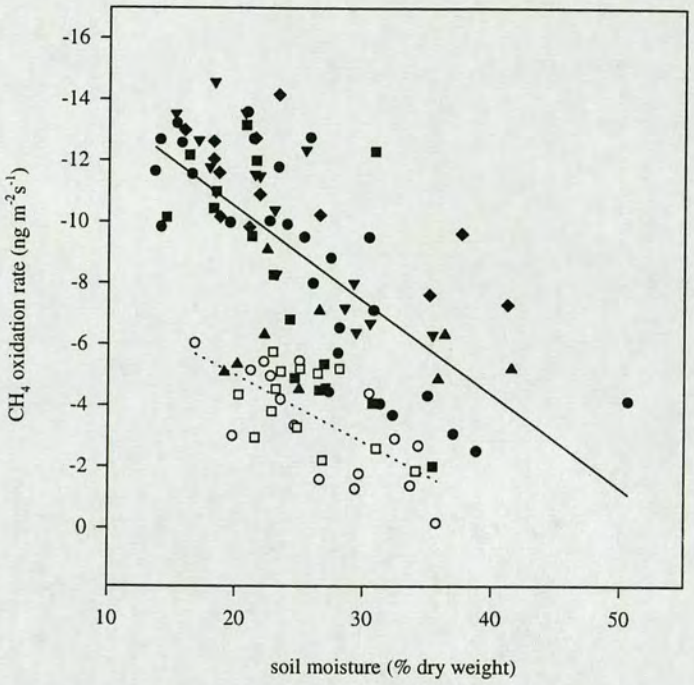

Figure. 5.6. The response of CH_4 oxidation rates to seasonal variation in soil water content at all sites investigated. The regression equation for the high diffusivity sites (▲ birch, ▼ far NH_3, ■ sitka, ● grass) CH_4 flux (ng m^{-2} s^{-1}) = -0.3*soil water content(%)+16.6 (r^2=0.469) and for the low diffusivity sites (□ near NH_3 and ○ alder) CH_4 flux (ng m^{-2} s^{-1}) = -0.2×soil water content (%)+9.3 (r^2=0.455).

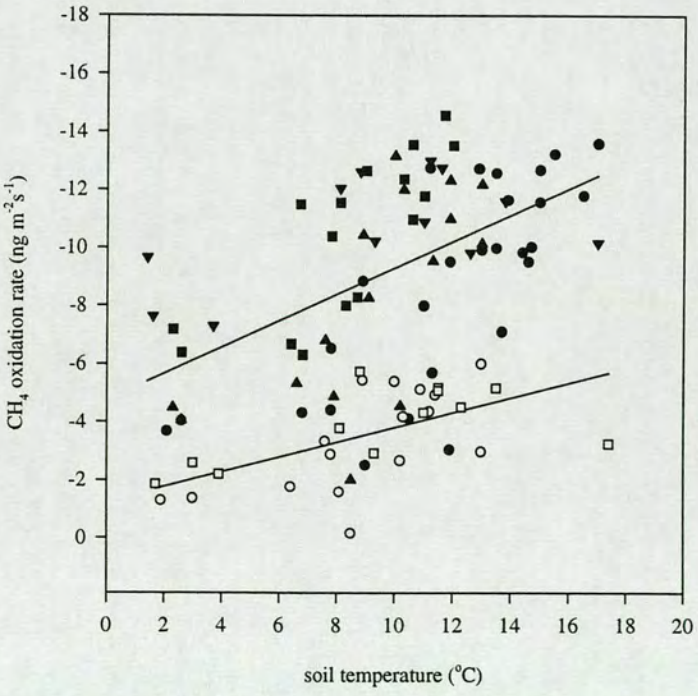

Figure 5.7. The response of CH_4 oxidation rates to seasonal variation in soil temperature at all the sites investigated. The regression equation for the high diffusivity sites (▲ birch, ▼ far NH_3, ■ sitka, ● grass) CH_4 flux (ng m^{-2} s^{-1}) = 0.4*soil temperature(°C)+4.8 (r^2=0.289) and for the low diffusivity sites (□ near NH_3 and ○ alder) CH_4 flux(ng m^{-2} s^{-1}) = 0.2×soil temperature(°C)+1.2(r^2=0.360).

The relationship between soil temperature and CH_4 oxidation rates gave a similar result, with the data showing two significant linear responses, dependent upon the soil diffusivity of the site (Figure 5.7). The high diffusivity sites increased in response to temperature at a rate of 0.4 ng m^{-2} $°C^{-1}$ ($r^2 = 0.222$, $p<0.05$), compared to 0.2 ng m^{-2} $°C^{-1}$ at the low diffusivity sites ($r^2 = 0.360$, $p<0.05$). Temperature responses were 56.0 kJ mol^{-1} at the low diffusivity sites and 39.4 kJ mol^{-1} at the high diffusivity sites, in good agreement with the temperature responses observed in other studies (Born *et al.*, 1991, Crill, 1991, Lessard *et al.*, 1994).

Best subsets regression indicated that soil water content explained the majority of the variability in CH_4 oxidation rates, particularly in the high diffusivity sites, where it explained 53.8% of the variability compared to only 28.9% explained by temperature. At the low diffusivity sites, again, most of the variability could be explained by soil water content, 47.2%, compared to 36.0% by soil temperature.

Rates of CH_4 oxidation at Glencorse and Devilla, which ranged from -0.2 to -18.8 ng m^{-2} s^{-1}, were at the low end of the range compared to those found in other studies. For example, Crill (1991) observed oxidation rates ranging from 0 to -40.5 ng m^{-2} s^{-1} from a mixed hardwood forest in the NE USA, Castro *et al.* (1995) measured oxidation rates between -13.9 and -69.0 ng m^{-2} s^{-1} from a range of forest soils in NE USA, and Dobbie *et al.*, (1996) measured oxidation rates ranging from -0.1 to -38.2 ng m^{-2} s^{-1} in a range of European soils. The annual average oxidation rate for the sites measured in this study was 2.3 kg ha^{-1} y^{-1}. Potter *et al.*, (1996) estimated that cool temperate forests oxidised 1.3 and 4.5 kg ha^{-1} y^{-1}, from a model computation and from extrapolation of measured fluxes, respectively. However, winter oxidation rates may have been underestimated in the model by setting fluxes from temperate forest to zero when several studies have shown significant winter fluxes (Crill, 1991, this study).

5.5.2 Factors affecting inter-site variability

Mean CH_4 oxidation rates from each site over the measurement period were significantly correlated with soil bulk density (Figure 5.4), which may be used as an indicator of soil diffusivity. The gaseous diffusion of CH_4 to methanotrophs is an important controlling factor in the magnitude of CH_4 oxidation rates (Dorr *et al.*, 1993) as methanotrophs are dependent on an adequate supply of CH_4 for energy and growth. As soil bulk density increases, so the diffusion of CH_4 to methanotrophs decreases, resulting in smaller rates of CH_4 oxidation. Dorr *et al.* (1993) demonstrated that soil gas diffusivity, as determined by

soil texture, affected CH_4 oxidation rates in a range of European soils. Keller & Reiners (1994) also observed that soil diffusivity determined CH_4 oxidation rates, and related increases in soil bulk density following recovery of cleared forest to increasing oxidation rates. In MacDonald *et al.* (1996)/ Chapter 3 short term measurements of a range of mineral soils, including Glencorse and Devilla, were significantly correlated with soil bulk density. From this study it appears that the relationship is also valid over yearly time-scales when the variability caused by seasonal changes in soil water content and temperature are taken into account.

5.5.3 The effect of N on CH_4 oxidation rates

5.5.3.1 Nitrogen fertilisation investigation, Glencorse

The fertilisation of the grass plot with NH_4NO_3 resulted in an average decrease in CH_4 oxidation rates of 23% over the measurement period. Inhibition of CH_4 oxidation rates by various forms of N have been documented in several studies (Steudler *et al.*, 1989, Crill *et al.*, 1994, Sitaula *et al.*, 1995). The decrease in oxidation rates observed in this study was in a similar range to inhibitions observed elsewhere (Mosier *et al.*, 1991, Steudler *et al.*, 1989, Sitaula *et al.*, 1995). However, significantly larger inhibitions, up to 95%, have also been observed (Adamsen & King, 1993, MacDonald *et al.*, 1997/Chapter 4). The mechanism responsible for the inhibition is not well understood and was discussed in detail in Chapter 4. Conflicting results between studies have resulted in several theories to explain the observed inhibition. Bedard & Knowles (1989) attributed the effect to a competitive inhibition of CH_4 oxidation, due to the similarity of the enzyme systems catalysing the initial stage of CH_4 and NH_4 oxidation. However, the inhibition has been observed to persist for long periods after added NH_4 has been nitrified (Mosier *et al.*, 1991, Willison *et al.*, 1995), and Mosier *et al.* (1991) suggested that N turnover rather than mineral N content may be affecting CH_4 oxidation rates. However, results have shown that salts commonly applied in N deposition studies can also significantly inhibit CH_4 oxidation rates (MacDonald *et al.*, 1997/Chapter 4); the mechanism of this inhibition is unknown. In general, field manipulation studies have not shown a clear relationship between the magnitude of the inhibition and the amount or the frequency of fertiliser application (Mosier *et al.*, 1991, Dunfield *et al.*, 1995). In this study, available NH_4-N and NO_3-N concentrations were elevated in the fertilised plot relative to the control plot. However, the degree of inhibition was not related to the concentration of soil available N concentrations. The various responses to N fertilisation/deposition in the literature raises questions about the

microorganisms involved. Willison *et al.*, (1995) speculated that changes in microbial populations may have occurred. The role of nitrifiers in CH_4 oxidation has been subject to much discussion (Topp & Hanson, 1991, King 1992, Hŋtsch *et al.*, 1993), but several authors have concluded that nitrifiers are not involved in methanotrophy (King, 1992, Hŋtsch *et al.*, 1994, Bender & Conrad, 1994, Sitaula *et al.* 1995). King & Schnell (1994) have suggested that the persistence of the inhibition may be related to the limited ability of methanotrophs to grow on atmospheric CH_4. These microorganisms have not yet been isolated, and therefore most of their properties are unknown. Different populations may have different levels of tolerance to N, or it is possible that small niches in the soil protect a proportion of the microorganisms from the effects of N. It should be noted that the sites at Glencorse were situated on ex-agricultural land which presumably had at some point been converted from forest. Deforestation and conversion to agriculture has been shown to inhibit CH_4 oxidation rates in several studies (Lessard *et al.*, 1994, Dobbie *et al.*, 1996), and recovery times after cessation of agricultural activity are long, > 100 years (Priemŏ *et al.*, 1997). Therefore, the oxidation rates observed at the Glencorse sites are probably significantly lower than if the site had not previously been disturbed. Studies have shown that further additions of N to systems already inhibited by N inputs, such as agricultural land, has no further inhibitory effect (Bronson & Mosier, 1993, Flessa *et al.*, 1995), therefore the inhibitory effect observed in this study may have been larger had the measurements been carried out in an undisturbed environment.

5.5.3.2 N fixing alder plot, Glencorse

Rates of CH_4 oxidation in the alder plot were significantly smaller than in the other plots at Glencorse. The low oxidation rates were attributed to both the high NO_3-N concentrations found in this plot, as a result of N fixation by alder species, and the low soil gas diffusivity. The correlation between mean CH_4 oxidation rates and soil bulk density at the sites studied are shown in Figure 5.4. The mean oxidation rate in the alder plot is the only point lying outside the 95% confidence interval and may be indicative of the additional inhibition of CH_4 oxidation rates due to N. Rates of CH_4 oxidation in the alder plot, from two measurement days in the spring of 1994, were discussed in MacDonald *et al.*, (1996)/Chapter 3. From this study it is apparent that the small rate of CH_4 oxidation, and the large concentration of soil NO_3-N observed, persisted throughout the year.

5.5.3.4 NH_3 deposition, Devilla

Significant differences in CH_4 oxidation rates were observed at Devilla, however, they were not related to N input, with the far NH_3 site showing the largest mean oxidation rate and the near NH_3 site the smallest. No significant differences in soil available NH_4-N or NO_3-N were found between sites despite the atmospheric NH_3 input, however, the inputs of N were very low. Results from throughfall collectors showed that on average 3.6 kg N ha^{-1} y^{-1} as NH_4 and 2.1 kg N ha^{-1} y^{-1} as NO_3 were deposited (J.N. Cape pers. com.). It is possible that the deposited N was taken up by the microbial biomass, or immobilised on cation exchange sites in the soil, or that there is a threshold value for N inhibition ie. under a certain concentration N input has no effect on the microbial community. The observed differences in CH_4 oxidation rates could be explained by variations in the diffusivity of the soil as indicated by soil bulk density. The differences in soil diffusivity may have masked any N effect.

5.6 Summary

Rates of CH_4 oxidation from two mineral forest soils showed significant seasonal variability. Soil water content exerted the dominant control over CH_4 oxidation rates, with soil temperature showing a lesser effect. Spatial variability in CH_4 oxidation rates was related to soil bulk density, indicating that the gaseous diffusion of CH_4 to methanotrophs was controlling CH_4 oxidation rates. N input was found to have an inhibitory effect on rates of CH_4 oxidation in an N fixing alder plantation, and N fertilisation of a grass plot inhibited CH_4 oxidation rates by 23%.

Chapter Six

Land use and seasonal effects on CH_4 flux

6.1 Abstract

Methane flux was measured from adjacent areas of peat bog, grazed grassland, a barley field and a shelter belt of deciduous and coniferous trees throughout 1995 in order to investigate the relationship between land use and seasonal effects on CH_4 flux. Both CH_4 oxidation and emission were observed at the agricultural and peat bog sites with fluxes ranging from -5.9 to 7.5 ng m^{-2} s^{-1}. At the shelter belt site net CH_4 oxidation was observed throughout the year with rates ranging between -1.3 and -8.5 ng m^{-2} s^{-1}. Oxidation rates in the agricultural soils were on average 70% lower than those in the shelter belt despite these soils being significantly drier, clearly showing the inhibitory effect of agriculture on CH_4 oxidation rates. CH_4 flux at all sites showed significant responses to variations in soil water content and temperature. CH_4 fluxes at the agricultural and peat bog sites were determined mainly by soil water content, while at the shelter belt site the rate of CH_4 oxidation was mainly determined by soil temperature.

6.2. Introduction

Land use has been shown to influence the magnitude and direction of CH_4 flux (Keller *et al.*, 1993, Lessard *et al.*, 1994, Dobbie *et al.*, 1996). Most published studies have focused on the effect of deforestation and conversion to agriculture, and have consistently shown a decrease in CH_4 oxidation rates (Keller *et al.*, 1993, Lessard *et al.*, 1994, Dobbie *et al.*, 1996), leading to speculation that a decrease in the soil sink for CH_4 may have contributed to the rise in atmospheric CH_4 concentrations (Ojima *et al.*, 1993, Dobbie *et al.*, 1996).

Seasonal variations in CH_4 oxidation rates have been observed in many soils (Crill, 1991, Lessard *et al.*, 1994) and long term studies are required for the accurate prediction of fluxes. The influence of soil water content and temperature changes on CH_4 oxidation rates was discussed fully in MacDonald *et al.* (1997)/Chapters 4 and 5. In short, soil water content has been observed to exert the dominant control on CH_4 oxidation rates in many soils (Lessard *et al.*, 1994, Castro *et al.*, 1995) by restricting the supply of substrate to CH_4 oxidisers. However, temperature is important in some soils, showing an affect on rates at low temperatures (Castro *et al.*, 1995), and also controlling seasonal variability in CH_4 oxidation rates when soil diffusivity is not limiting (MacDonald *et al.*, 1997/Chapter 4).

Methane emission from wetlands generally shows a strong seasonal response to changes in water table and temperature (Dise, 1993, Roulet *et al.*, 1993), with water table fluctuations exerting the dominant influence on rates of CH_4 emission by influencing the degree of anaerobicity. CH_4 oxidation in the surface layer of wetlands also controls net emission rates (Oremland & Culbertson, 1992), therefore conditions which affect oxidation rates also affect the net CH_4 emission.

In this study CH_4 flux was measured throughout 1995 from four adjacent land use types: peat bog, grazed grassland, barley field and shelter belt. The objective of the measurements was to investigate the effects of land use and environmental parameters on a seasonal basis on CH_4 flux. Plate 6.1 shows the field site with the shelter belt adjacent to the arable field and the peat bog in the background.

6.3. Materials and Methods

6.3.1. Site Description

Methane fluxes were measured at Springfield Farm, Penicuik, Midlothian (NT 221562) about 20 km SW of Edinburgh, Scotland, in adjacent areas of a peat bog, grazed grassland, barley field and shelter belt.

Plate 6.1 The shelter belt adjacent to the arable field with the peat bog in the background at the Springfield Farm field site.

The peat bog was ombrotrophic with an average peat layer of 60 cm and consisted of unimproved, unfertilised moorland vegetation, grazed all year round by sheep and horses with a very low animal density. The peat bog had been partially drained approximately 40 years ago and as a result very few pools of open water were present. The vegetation was dominated by a mixed carpet of *Sphagnum* species, *Deschampsia flexuosa*, *Molina caerulea*, *Eriophorum vaginatum*, *Festuca ovina*, *Nardus stricta*, *Vaccinium myrtillus* and *Carex* species.

The grazed grassland, barley field and shelter belt were on an organic soil (Brown & Shipley, 1982), presumably originally reclaimed from the peat bog. The grass field was fertilised at the beginning of April with chicken manure and was grazed with horses and cattle between June and October. The barley field was ploughed before the February sampling. It was sown with spring barley at the beginning of April and rolled before the next sampling in May. It was fertilised with NH_4NO_3 (88 kg N ha^{-1}) during May. The shelter belt seperated the grazed grassland from the barley field. The tree species present included Scots pine, oak and beech.

6.3.2 Field measurements of CH_4 flux

Fluxes were measured using the static chamber technique as described in Chapter 2. Three chambers were installed at each site and fluxes were measured at approximately monthly

intervals between February and October 1995. Soil samples were routinely taken on each measurement day and analysed for water content, pH, available NO_3-N, available NH_4-N, bulk density and organic matter content as described in Chapter 2.

6.3.3. Statistical analysis

Methane fluxes from each of the three chambers were averaged for each measurement day. Correlations were then carried out using the Pearson product moment correlation coefficient between the fluxes at each site and the environmental variables measured. The significance of differences in fluxes between sites were determined using an analysis of variance (ANOVA).

6.4. Results

6.4.1 Methane flux measurements

Both net CH_4 oxidation and emission were observed, with fluxes ranging from -8.5 to 7.5 ng m^{-2} s^{-1}. The peat bog, grazed grass and barley field sites showed fluxes which were not significantly different ($p<0.05$) from each other throughout the year. The largest rate of CH_4 emission at 7.5 ng m^{-2} s^{-1} was observed at the peat bog site, whereas the largest rate of oxidation at -8.5 ng m^{-2} s^{-1} was found at the shelter belt site where CH_4 oxidation was consistently observed throughout the year. Mean oxidation rates over the measurement period from the agricultural sites were 70 % lower than those observed in the shelter belt. Mean CH_4 fluxes and the soil parameters measured are shown in Table 6.1. The seasonal variation in CH_4 flux at each site is shown in Figure 6.1. Soil water content and temperature were significantly correlated with each other at all sites ($p<0.05$).

At the peat bog site both CH_4 oxidation and emission was observed. Measured fluxes ranged from -5.9 to 7.5 ng m^{-2} s^{-1} and showed a significant linear correlation with soil water content ($r^2=0.841$, $p<0.001$). No significant correlation with soil temperature was observed. The direction of flux changed from emission to deposition when the soil water content was approximately 380%. Due to the high spatial variability between chambers on each measurement day soil water content was measured at each chamber site. The variability between chambers was found to be related to the soil water content. Chamber 1, with the largest soil water content, had the largest emission flux and showed a larger variability throughout the year, than chambers 2 and 3, which showed similar small fluxes despite having different water contents (Figure 6.2). When the relationship between soil water content and and CH_4 flux was examined in more detail, by examining the relationship at

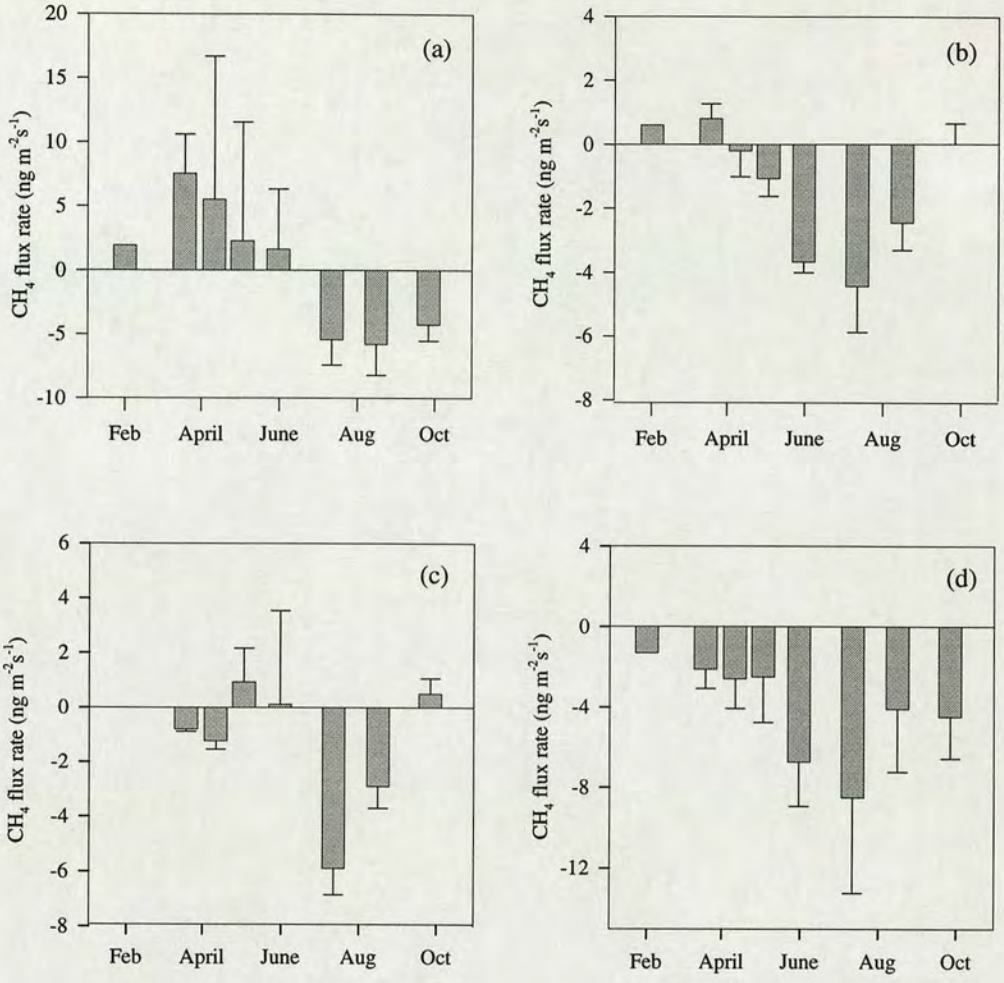

Figure 6.1. Seasonal variation in CH_4 flux, measured between Feb and Oct 1995 at **a)** the peat bog site, **b)** the grazed grass site, **c)** the barley field and **d)** the shelter belt site. Error bars represent the standard deviation from the mean.

each chamber as opposed to the average fluxes of the three chambers, the response to soil water content showed an exponential increase ($r^2 = 0.394$, $p<0.05$) (Figure 6.3).

The agricultural sites showed small CH_4 fluxes, ranging from -4.4 to 0.8 ng m^{-2} s^{-1} in the grazed grass site and from -5.9 to 0.5 ng m^{-2} s^{-1} in the barley field (Figure 6.1). CH_4 flux was significantly linearly correlated with soil water content in the barley field ($p<0.05$, $r^2=0.528$). At the grazed grass site CH_4 flux was significantly linearly correlated with soil water content and soil temperature. A best subsets regression showed that soil water content showed the most significant correlation with CH_4 flux ($r^2=0.889$, $p<0.001$) relative to soil temperature ($r^2=0.607$, $p<0.05$).

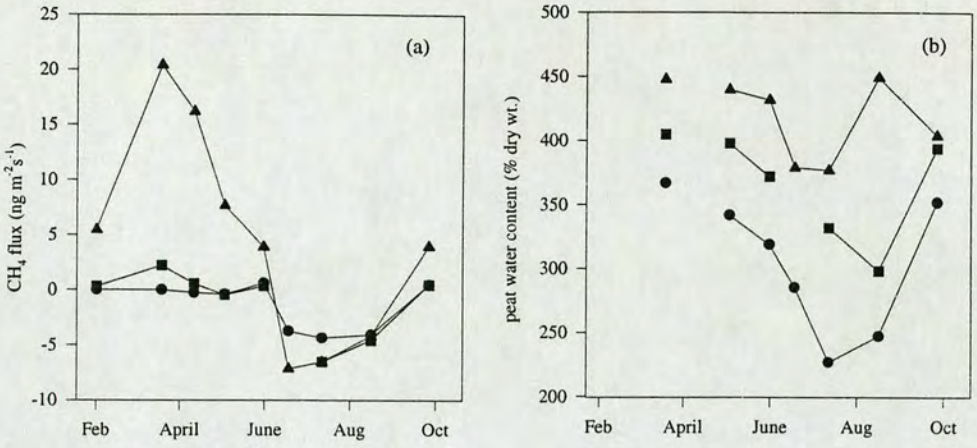

Figure 6.2. a) CH_4 flux and **b)** peat water content from chamber 1 (▲), chamber 2 (●) and chamber 3 (■) at the peat bog site.

The largest CH_4 oxidation rates were observed throughout the year at the shelter belt site and ranged from -1.3 to -8.5 ng m^{-2} s^{-1} (Figure 6.1). CH_4 oxidation rates were correlated both with soil temperature and soil water content. A best subsets regression showed that soil temperature showed the strongest relationship (r^2=0.746, $p<0.001$) (Figure 6.4), a slightly weaker correlation was observed with soil water content (r^2=0.631, $p<0.05$). An Arrhenius plot gave an activation energy of 88.6 kJ mol^{-1}.

6.4.2 Soil parameters

Soil available NH_4-N concentrations showed a seasonal variation with a summer maxima, at all sites. The largest concentrations were observed in the barley field in response to fertilisation. Significant NO_3-N concentrations throughout the year were only observed in the barley field. Soil pH was largest in the agricultural fields, and in the barley field varied between 4.5 and 6.2, in response to regular liming. Measurements of water table depth at the peat bog site (J. Moxley pers. comm.) indicated that during August the water table was between 23 and 30 cm below the surface, whereas in winter it was between 0 and 10 cm. Soil parameters are shown in Table 6.1. Soil bulk density was lower and organic matter content was higher in the shelter belt than in the agricultural soils.

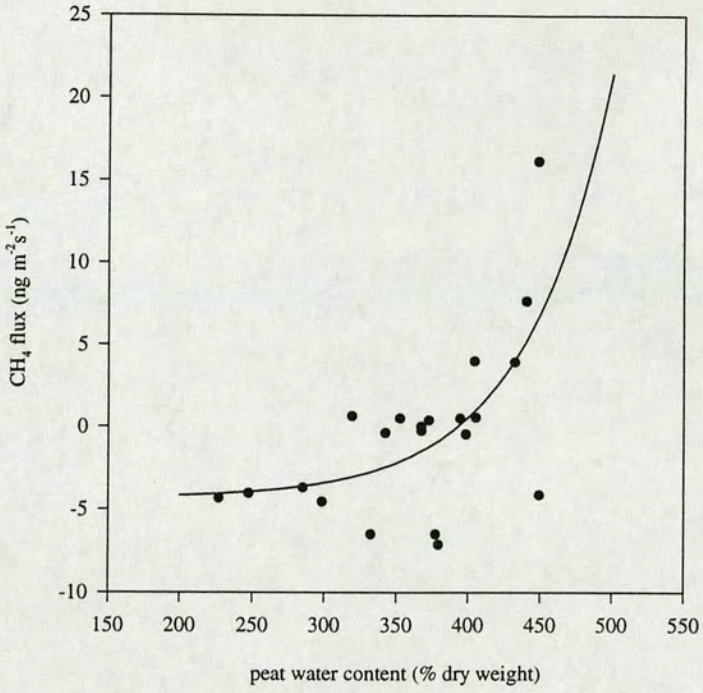

Figure 6.3. The response of CH_4 emission from each chamber to content at the peat bog site between Feb and Oct 1995. An exponential curve was fitted (r^2 0.394, $p<0.05$) and the regression equation was; CH_4 flux = $6.5e^{-3}$*exp($1.7e^{-2}$)*water content (%)-4.

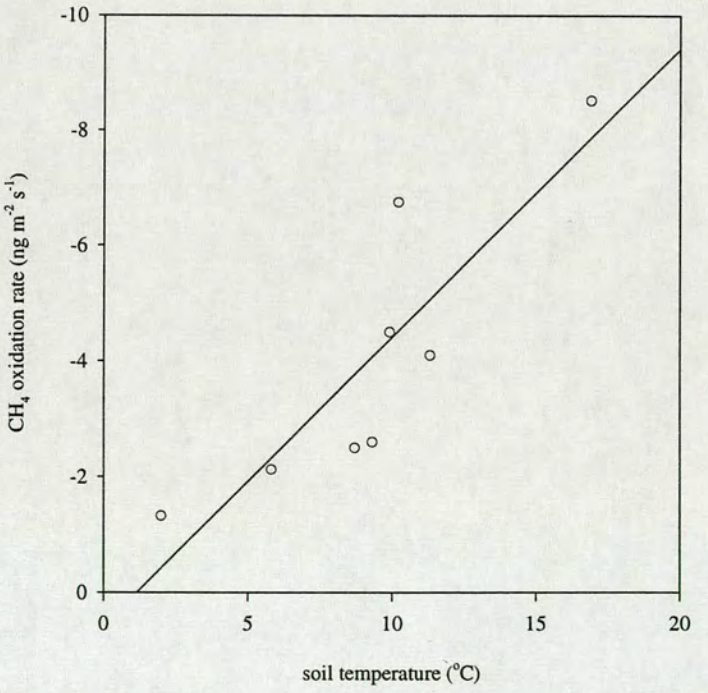

Figure 6.4. The temperature response of CH_4 oxidation rates at the shelter belt site. The regression equation was CH_4 flux (ng m^{-2} s^{-1}) =-0.5*temperature(°C)+0.578 (r^2=0.745, $p<0.001$).

Table 6.1. CH_4 flux and soil characteristics from the peat bog, grazed grass, barley field and shelter belt sites.

Site	Peat bog	Grazed grass	Barley field	Shelter belt
mean CH_4 flux ($ng\ m^{-2}\ s^{-1}$)	0.8 (6.4)[a]	-1.3 (2.0)	-1.1 (2.5)	-4.0 (3.3)
mean soil water content (% dry wt.)	382.1 (50.4)	46.3 (10.3)	40.6 (14.0)	82.1 (29.5)
mean soil temperature (°C)	10.4 (3.8)	10.3 (4.4)	10.9 (5.1)	9.3 (4.3)
mean NO_3-N (:g g^{-1} dry soil)	0	0.1 (0.2)	9.3 (9.9)	0
mean NH_4-N (:g g^{-1} dry soil)	12.0 (8.2)	10.1 (2.5)	9.1 (9.1)	9.3 (5.1)
pH	3.2	5.6	5.4	3.5
organic matter (%)	nd	17	14	31
bulk density ($g\ cm^3$)	nd	0.7	0.8	0.4

nd = not determined, [a] = mean, ( ) standard deviation

6.5. Discussion

Methane oxidation rates from the shelter belt soil were low compared to other studies in temperate forests (Crill, 1991, Dorr *et al.*, 1993, Dobbie *et al.*, 1996). Conditions such as high soil water content, low temperature and low pH may not have been conducive to producing the larger fluxes observed elsewhere. Low oxidation rates similar to those observed in the barley and grazed grassland soils have been observed from other agricultural systems (Lessard *et al.*, 1994), and net CH_4 emission was also observed from an agricultural soil in Scotland (MacDonald *et al.*, 1996). The CH_4 flux from the peat bog was also at the low end of the range observed from other peatlands (Bartlett *et al.*, 1993) probably as a result of the drainage that had occurred in the past.

6.5.1. Effect of land use

Land use and its associated effects on soil conditions determined the magnitude and variability of CH_4 flux at each site (Table 6.1). The mean oxidation rates in the agricultural soils were about 70% smaller than those in the adjacent shelter belt, an amount similar to the

levels of inhibition observed in similar studies (Ojima *et al.*, 1993, Dobbie *et al.*, 1996). Soil water content has been shown to determine CH_4 flux in many studies (Lessard *et al.*, 1994, Dunfield *et al.*, 1995, Castro *et al.*, 1995). However, in this study the effect of land use confounded any soil water content effect and the relationship between mean CH_4 flux and mean soil water content between sites was not linear (Figure 6.5). High soil water content clearly determined the net CH_4 emission observed at the peat bog. At the other three sites the largest oxidation rates were observed in the shelter belt despite the mean soil water content at this site being approximately double that at the grazed grass and barley field sites (Table 6.1). Therefore other factors must have been controlling oxidation rates. The conversion of forest to agriculture has been shown to decrease CH_4 oxidation rates in many studies (Keller *et al.*, 1993, Lessard *et al.*, 1994, Dobbie *et al.*, 1996), but the mechanisms responsible for this inhibition are not well understood. Compaction due to tractor traffic has been shown to decrease oxidation rates by up to 50% (Hansen *et al.*, 1993), presumably by decreasing the diffusion of CH_4 substrate to microorganisms, and MacDonald *et al.* (1996/Chapter 3) observed a positive correlation between soil bulk density and CH_4 oxidation rates from a range of mineral soils. The large bulk densities observed in the grazed grass and barley field soils relative to those in the shelter belt (Table 6.1) may be indicative of a diffusional limitation on oxidation rates at these sites caused by agricultural practices. Disturbance to the soil profile has also been indicated as an inhibitor of CH_4 oxidation rates (Lessard *et al.*, 1994, Goulding *et al.*, 1995), however, Priemé *et al.*, (1997) suggested that changes in microbial numbers or enzyme activity, as well as physical disturbance, may be responsible for the inhibition. Fertilisation with various N compounds has been observed to inhibit CH_4 oxidation rates (Steudler *et al.*, 1989, Adamsen & King, 1993, Goulding *et al.*, 1995). The mechanism responsible for the inhibition of oxidation rates by N is not yet fully understood. Bedard & Knowles (1989) have suggested that competition between CH_4 and NH_4 for the active sites on the monooxygenase enzyme system which catalyses the first step in the oxidation of CH_4 and NH_4 is responsible. However, the results from many studies show conflicting evidence (Dunfield & Knowles, 1995, Wilison *et al.*, 1995). Inhibition of CH_4 oxidation rates by N compounds was discussed fully in MacDonald *et al.* (1996) and in Chapter 4. In this study the largest soil NH_4-N and NO_3-N concentrations coincided with a net CH_4 emission from the barley field following fertilisation, and in the grazed grassland the largest CH_4 emission was observed in April following fertilisation with chicken manure, despite a 4% decrease in soil water content and a 4.4 °C increase in soil temperature. It is possible that fertilisation had decreased the oxidation capacity of the soil to such an extent

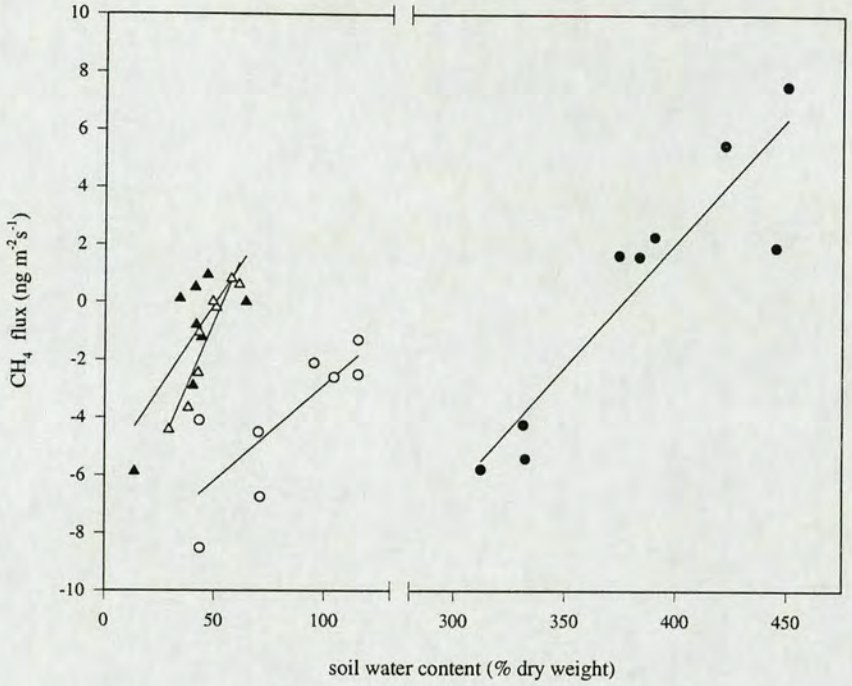

Figure 6.5. The response of CH_4 flux to soil water content at the barley field (▲), the grazed grassland (Δ), the shelter belt (○) and the peat bog (●).

that net emission from zones of production in anerobic microsites had occurred, or that the input of organic matter from the chicken manure plus the rise in temperature induced new anaerobic zones resulting in net emission. A similar observation was made following fertilisation of a winter wheat field (MacDonald *et al.*, 1996) and was discussed in Chapter 3. However, available NH_4-N concentrations in the soil were not significantly different between the shelter belt soil and the agricultural soils, indicating that inhibition of CH_4 oxidation was not related to available NH_4-N concentration in the soil. Soil available NO_3-N was only detectable in the agricultural sites and may be indicative of nitrification of the added N. Sitaula *et al.* (1993) also found no correlation between NH_4-N concentration and CH_4 oxidation rates, and MacDonald *et al.* (1997) found a positive correlation between soil NH_4-N concentrations and CH_4 oxidation rates. Mosier *et al.* (1990) speculated that nitrification rate rather than NH_4 concentration was responsible for the inhibition.

6.5.2. Seasonal variation in soil water content, temperature and CH_4 flux

At the peat bog site soil water content determined the magnitude and direction of CH_4 flux. As the peat dried and the water table dropped over the summer months, CH_4 emission

steadily decreased until net CH_4 oxidation was observed in July. As the water table drops, an aerobic zone is formed, and much of the CH_4 produced may be oxidised before reaching the atmosphere. Methanotrophs have been estimated to consume up to 80 % of CH_4 produced from some wetlands (Oremland & Culbertson, 1992). Net CH_4 emission was again observed in October when the soil water content had returned to winter values, accompanied by a resultant decrease in redox potential and population growth and establishment of the methanogenic population. Soil water content or water table depth has been recognised as the primary contolling factor affecting CH_4 emission rates in several studies (Roulet *et al.*, 1993, Freeman *et al.*, 1993, Moore & Dalva, 1993), by being the primary influence over the degree of anaerobicity, an essential precursor of methanogenesis. The variability in flux between chambers at the peat bog site was mostly a result of soil water content. However, high spatial variability is characteristic of peatland CH_4 emissions (Crill *et al.*, 1988, Dise, 1993) and several other factors can influence CH_4 flux, including substrate quality and botanical composition of peat (Nilsson & Bohlin, 1993, Dise 1993, Martikainen *et al.*, 1995). The transport of CH_4 through vascular plants (Whiting & Chanton, 1992), also strongly affects net CH_4 flux by providing a bypass through the aerobic zone. An analysis of CH_4 emission and plant species composition and biomass was beyond the scope of this study.

The strong relationship between CH_4 production and temperature has been well established (Svensson, 1984, Dunfield *et al.*, 1993) and is discussed fully in Chapter 7. However, the relationship between net CH_4 flux and soil temperature was not significant at this site due to the dominant effect of soil water content, and the different temperature dependencies of the methanogenic and methanotrophic microbial populations. However, CH_4 emission did increase by a factor of 3 as temperature increased from 2.7 to 6.3 °C between the February and April sampling periods while the soil water content was roughly constant. Most of the variability occurred in chamber 1, indicating that the strong temperature response occurs only if soil water content is not limiting. However, despite further increases in temperature, CH_4 emission decreased as the peat dried out and the peat eventually became a net sink, demonstrating the primary controlling influence of peat water content.

At the agricultural sites CH_4 flux appeared to be mainly controlled by soil water content, however, the correlation was weaker at the barley field than at the grazed grassland site, probably as a result of agricultural practices such as ploughing, fertilisation and rolling, overshadowing soil water content and temperature effects. CH_4 oxidation rates were generally low at these sites. The exception was the relatively large rate of oxidation

observed in August in the barley soil which coincided with a very low soil water content 13.6% (dry weight) in the top 10 cm, which in this organic soil may have allowed the diffusion of CH_4 deep into the soil profile where methanotrophs were not inhibited by disturbance or fertilisation. The agricultural soils had higher bulk densities than the shelter belt soil, as a result of the agricultural practices. The subsequent decrease in pore space, while limiting the transport of CH_4 to methanotrophs, would also have resulted in the soil air filled pore space becoming saturated more quickly following rainfall, the effect being an increase in the sensitivity of CH_4 oxidation rates to soil water content. Temperature responses of the methanotrophs would then only be significant when air filled pore space was not limiting, with the end result being that soil water content was the primary controlling factor affecting CH_4 oxidation rates in the cultivated soils.

In contrast to the agricultural sites, CH_4 oxidation rates in the shelter belt appeared to be most strongly influenced by soil temperature, with soil water content showing a less significant influence. Temperature has been observed to have a minor influence on CH_4 oxidation rates in many studies (Dorr *et al.*, 1993, Lessard *et al.*, 1994,) due to the diffusional control on the transport of CH_4 to methanotrophs as exerted by soil texture and soil water content (Dorr *et al.*, 1993, Castro *et al.*, 1995). However, if the porosity of the soil is such that changes in soil water content do not affect the diffusion of CH_4 to methanotrophs then other factors which can affect the rate of microbial activity, such as temperature, can have a contolling influence on CH_4 oxidation rates. A similar response was observed by MacDonald *et al.* (1997) in an upland peat and was discussed fully in Chapter 4.

The different responses to season observed in the grazed grass and barley field sites compared with those in the shelter belt soil were most likely due to the agricultural practices. Compaction due to cultivation and grazing resulted in a higher soil bulk density, which may have changed the seasonal response from one dependent on temperature to one dependent on diffusivity as determined by soil water content.

6.6 Summary

The different land uses showed distinct CH_4 fluxes as a result of soil physical and chemical characteristics. Rates of CH_4 oxidation from agricultural soils were on average 70% smaller than those from an adjacent shelter belt. Despite the soil water content in the shelter belt being double that in the agricultural soils, other soil parameters (bulk density and organic matter) were indicative of a textured porous soil. In contrast, in the agricultural sites

cultivation had resulted in soil with much smaller organic matter contents and higher bulk densities, resulting in a less efficient transport of substate to methanotrophs and hence smaller CH_4 oxidation rates. Net CH_4 emission was also occasionally observed from the agricultural soils. Fluxes of CH_4 from the four land use types showed seasonal variation in response to soil water content and temperature. At the peat bog site soil water content determined the magnitude and direction of flux. At the shelter belt the rate of CH_4 oxidation was mainly determined by soil temperature.

Chapter Seven

Factors affecting CH_4 emission rates from wetlands

(Data from this chapter are included in: Methane emission rates from northern wetlands; response to temperature, water table and transport, with D. Fowler, K.J. Hargreaves, U. Skiba, I.D. Leith & M.B. Murray as co-authors, Atmospheric Environment, in press.)

7.1 Abstract

Measurements of CH_4 flux were made from a range of micro-environments, along a soil water content gradient in an area of blanket bog in NE Scotland, and at an upland moorland site in NW England. CH_4 flux covered a wide range, the largest rate of CH_4 emission, at 780.4 ng m^{-2} s^{-1}, was observed from the common vascular plant *Menyanthes trifoliata* in a pool area of blanket bog in NE Scotland. Net CH_4 oxidation was observed from several of the drier sites in NW England, on which the dominant vegetation consisted of *Festuca* spp., *Carex* spp., *Nardus stricta* and *Juncus* sp. A significant temperature response was observed during the winter sampling period at the NW England site and activation energies of 56.1 and 34.8 kJ mol^{-1} were observed. The influence of soil temperature on CH_4 emission rates from intact peat monoliths, cored from the blanket bog in NE Scotland, was examined under controlled conditions and an exponential increase in response to increasing temperatures between 5 and 30 °C was observed with activation energies between 51.8 and 73.2 kJ mol^{-1}. The response of CH_4 emission through *Menyanthes trifoliata* to stomatal conductance was investigated under controlled conditions, and was found to have no significant effect on emission rates. The effect of S (100 kg S ha^{-1}) deposition to peat was investigated and was found to inhibit rates of CH_4 emission by up to 50%.

7.2 Introduction

Wetlands are the largest source for tropospheric CH_4 and global estimates cover a wide range currently between 55 and 150 Tg y^{-1} (IPCC, 1994). CH_4 emission from wetlands is characterised by high spatial and temporal variability (Roulet *et al.*, 1993, Dise *et al.*, 1993) and is affected by a wide variety of environmental variables including water table height, temperature, substrate availability, the presence of alternative electron acceptors, and the transport mechanism of CH_4 release to the atmosphere. The primary controlling factor is water table height (Dise *et al.*, 1993, Moore & Dalva, 1993) which affects the degree of anaerobicity of the peat, an essential requirement of methanogenesis, and also affects the depth of the aerobic layer and hence the oxidising capacity of the peat. Up to 80% of the CH_4 produced in wetlands has been estimated to be oxidised before reaching the atmosphere (Oremland & Culbertson, 1992). Previous work on intact peat monoliths (MacDonald *et al.*, 1997) established the importance of water table height on the flux of CH_4, with an order of magnitude difference between pool cores (surface water table) and hummocks (water table > 15 cm below the surface). Soil temperature also strongly affects the rate of methanogenesis and can significantly influence seasonal variations in flux (Williams & Crawford, 1984, Crill *et al.*, 1988). Measurements of CH_4 emission from peat monoliths over 18 months showed a significant linear response to temperature, with an approximate doubling of emission for a 10 °C increase in temperature (Fowler *et al.*, 1994). Electron acceptors such as NO_3^- and SO_4^{2-} are preferentially used as oxidants in the decomposition of organic matter before CO_2 reduction to CH_4 can begin (Conrad, 1989) and the inhibition of methanogenesis by SO_4^{2-} reducing bacteria competing for substrates has been observed in several studies (Westermann & Ahring, 1987, Nedwell & Watson, 1995). However, the effect of S inputs on the net flux of CH_4 from wetlands has not been fully investigated or quantified. The transport of CH_4 from zones of production, through vascular plants (macrophytes), to the atmosphere can affect the net flux of CH_4, by bypassing oxic zones where the CH_4 would otherwise be oxidised. Plant-dependent transport of CH_4 is therefore an important pathway and has been estimated to account for up to 90% of the emission from wetlands (Whiting & Chanton, 1992, Yavitt & Knapp, 1995, Shannon *et al.*, 1996). Substrate quality and the botanical composition of the peat have also been shown to influence CH_4 production (Nilsson & Bohlin, 1993, Martikainen *et al.*, 1995).

In order to investigate the environmental parameters affecting rates of CH_4 emission from wetland environments, measurements of CH_4 flux were made during field campaigns in the blanket bogs of Caithness, NE Scotland and from representative vegetation

communities at three altitudes at Great Dun Fell, NW England. Processes affecting CH_4 flux rates were then investigated under controlled conditions at ITE Edinburgh. The effects of temperature and sulphate were investigated using peat monoliths in controlled environment cabinets (CONVIRONS). The transport of CH_4 through *Menyanthes trifoliata* (bog bean) was investigated both in the field and under controlled conditions.

7.3 Materials and Methods

7.3.1 Site Description and field measurements

Loch More - Field measurements were made at Loch More (ND 065453), an area of blanket bog in The Flow Country, Caithness, during a three week field campaign in May 1994. The peat monoliths were cored from Loch Calluim (ND 023511), an area similar in vegetation type and topography to Loch More. The area of study represented a broad range of micro-environments, ranging from a relatively dry area of bog heather moor to an open pool area, where pools represented up to 30% of the land cover. Measurements of CH_4 flux were made using the static chamber technique described in Chapter 2. Measurements were made in areas with and without *Menyanthes trifoliata* in order to investigate their contribution to the total CH_4 flux. Sites were selected to cover a range of water table heights and vegetation types and are described in Table 7.1.

Great Dun Fell (NY 710322) is one of a range of hills in the North Pennines in NW England. Flux measurement were made in March and July 1995. This study site has been subject to many investigations, in particular into the effect of altitude on cloud, rainfall composition and cloud chemistry (eg. Fowler *et al.,* 1988). The vegetation was typical of an upland moorland community, and consisted predominantly of *Carex* spp., *Festuca* spp., *Eriophorum* spp., *Nardus stricta*, *Sphagnum* spp., and *Gallium saxatile*. The hill was grazed, for most of the year, by sheep. The soil was predominantly peat, of varying depths. Chambers were installed in a range of vegetation types at three altitudes, 440, 670 and 847 m. Flux measurements were carried out in March and then again in July 1995; the chambers were removed between sampling periods. Measurements were made using the static chamber technique as described in Chapter 2. Soil physical and chemical characteristics were determined using the methods described in Chapter 2. Sites were selected at each altitude to represent typical vegetation and peat water contents. Sites are described in Table 7.2 according to vegetation type, altitude and peat and water table depth.

Table 7.1. CH_4 flux and site characteristics from the sites studied at Loch More, Caithness, May 1994.

Site	Dominant Vegetation	Water Table (cm below surface)	Mean CH_4 flux (std. dev.) ($ng\ m^{-2}\ s^{-1}$)	n
Bog heather moor	*Calluna vulgaris*	> 100*	6.7 (10.9)	40
Pool edge	*Eriophorum* spp., *Sphagnum* spp. *Calluna vulgaris*	> 10	77.8 (35.8)	5
Hollow	*Sphagnum* spp., *Menyanthes trifoliata*	surface	572.7 (506.9)	6
Pool -open water	-	**	64.4 (29.3)	4
Pool-bog bean	*Menyanthes trifoliata*	**	780.4 (73.4)	5

* peat depth was greater than 100 cm and water table was below subsoil.
** pool depths estimated to be between 20 and 100 cm

7.3.2 Peat monoliths

Process based investigations were carried out on intact peat monoliths, which had been collected using 30 cm diameter cylindrical aluminium coring devices in 1992, from an area of blanket bog near Loch Calluim in Caithness. Peat monoliths were kept in plastic buckets (30 cm in diameter, 40 cm deep), in open top chambers (OTC) and glasshouses, and were watered with deionised water. The use of these large monoliths allowed a close approximation of field conditions to be made, as they were large enough to maintain the peat structure and vegetation while allowing experiments to be carried out under controlled conditions. The monoliths used in the temperature response investigation were from pools (or hollows) with a surface water table and vegetation which was mainly comprised of *Sphagnum* spp., *Eriophorum* spp. and *Menyanthes trifoliata*.

Table 7.2 Site Description, Great Dun Fell.

	Dominant Vegetation	Altitude (m)	Approximate Peat Depth (cm)	Approximate Water table depth (cm)
Fell Gate grass	*Festuca* spp., *Trifolium repens*	440	~20	-
Fell Gate *carex*	*Carex* spp., *nardus sticta, Gallium saxatile*	"	"	-
Fell Gate *carex* pool**	*Carex* spp., *Sphagnum* spp., *Gallium saxatile*	"	"	-
Fell Gate *carex/sphagnum*	*Sphagnum* spp., *Carex* spp., *Polytrichum* spp., *Mnium* spp.	"	"	-
Fell Gate *sphagnum* pool**	*Sphagnum* spp.	"	"	-
Fell Gate mud pool**	-	"	"	-
Fell Gate *juncus**	*Juncus* spp. *Gallium saxatile*	"	-	-
Mine Road hill *nardus*	*Nardus sticta, Festuca* spp., *Gallium saxatile*	670	<100	-
Mine Road hill *juncus*	*Juncus* spp. *Gallium saxatile*	"	-	-
Mine Road *nardus*	*Nardus sticta, Carex* spp., *Polytrichum* spp., *Gallium saxatile*	"	<100	26
Mine Road *juncus**	*Juncus* spp.	"	-	-
Mine Road *Eriophorum*	*Eriophorum* spp., *Carex* spp.	"	>100	36
Summit grass	*Festuca* spp., *Gallium saxatile*	847	10-100	-
Summit *carex/nardus*	*Carex* spp., *Festuca* spp.	"	"	-
Summit *carex* pool**	*Carex* spp., *Eriophorum* spp.	"	"	23
Summit *carex sphagnum*	*Sphagnum* spp., *Carex* spp.	"	"	-
Summit *sphagnum* pool**	*Sphagnum* spp.	"	"	-

* *Juncus* sp. was only apparent in areas with soil at surface.
**These sites (Fell Gate *carex* pool, *sphagnum* pool , mud pool, Summit *carex* pool and *sphagnum* pool) were dry pools which were water filled during the winter.

7.3.3 Temperature response of CH_4 emission from pool monoliths

Peat monoliths (n=3) were maintained in CONVIRONS under constant conditions of humidity (70%) and light (300 μE m^{-2} for 12 hours day^{-1}). Flux measurements were made (n=4 day^{-1}) using a static chamber technique similar to the method described in Chapter 2. The chamber which was sealed to the monolith bucket consisted of two sections, an extension piece to avoid damage to the vegetation, and a chamber containing a fan to promote circulation throughout the chamber. The total headspace volume of the chamber was 0.02 m^3. Samples (100 ml) were withdrawn at time 0 into a tedlar bag using a small pump. The chamber was left in place for between 10 and 15 minutes before the headspace was again sampled. Routine tests were made to ensure the CH_4 emission was linear (Chapter 2) over the time period, and replicated measurements over different time periods showed the method was reproducible. Measurements where the ambient samples were found to be significantly larger than 1.8 ppm were discarded because of the possibility that disturbance during chamber installation had released bubbles containing high concentratioms of CH_4. Gas samples were analysed by TDL spectroscopy. Flux measurements were made in the dark in order to avoid heating effects by the lights. To investigate the temperature response, the monoliths were maintained successively at 5, 10, 15, 20, 25 and 30 °C for 24 hours. Peat temperature was monitored using a temperature probe at 4 depths (0, 5, 10, 20 cm) which showed that it took approximately 15 hours for the whole core to equilibrate to each temperature. Following this investigation the monoliths were maintained at 15 °C.

7.3.4 The effect of sulphate application on CH_4 emission rates

In the first experiment two monoliths from the OTC, whose temperature responses had been well characterised (Fowler *et al.*, 1994) and had similar vegetation, were selected. Sulphate was applied at a rate of 100 kg S ha^{-1} as Na_2SO_4 in 500 ml of deionised water. The control monolith received 500 ml of deionised water only. Flux measurements were made on a daily basis using the chamber method described above.

For the second experiment the monoliths that had been used for the temperature response were maintained in the CONVIRON at 15 °C and 300 μE m^{-2} 12 hours/day. S was applied at a rate of 100 kg ha^{-1} as $(NH_4)_2SO_4$ and Na_2SO_4 in 500 ml of deionised water. The control monolith received 500 ml of deionised water only. Flux measurements were made on a daily basis using the chamber method described above.

7.3.5 Measurements of CH_4 transport through Menyanthes trifoliata

The vascular transport of CH_4 was further investigated using peat monoliths in the OTC and CONVIRON. A method was developed using an infra-red CO_2 gas analyser (Li-Cor, Lincoln, NE, USA) to enable the measurement of stomatal conductance and CH_4 emission from individual leaves of *Menyanthes trifoliata*. Leaves (or stems or whole plants) were enclosed within a cuvette through which ambient air was circulated around a closed loop with a total volume of 1 l. Stomatal conductance readings were made (M. Murray, ITE Edinburgh) within one minute of closing the cuvette. The cuvette was then flushed with ambient air, and closed again to allow the CH_4 measurement to be made. Samples were withdrawn using 5 ml gas tight syringes (Hamilton) after 0 and 5 minutes. Enclosure time was minimised to avoid varying CO_2 concentrations and stomatal conductance. Relative humidity was kept constant by adjusting the flow of the gas through a dessicant. CH_4 samples were analysed by GC. CH_4 emission was measured from a range of plants in peat monoliths in OTC's. The effect of light was investigated by measuring CH_4 emission and stomatal conductance from plants in peat monoliths in dark and light conditions at both 10 °C and 20 °C. The physiology of *Menyanthes trifoliata* was investigated (K. Ingleby, ITE Edinburgh) by taking thin films and cross sections of leaves and stems and examining them under a compound microscope.

7.4 Results

7.4.1 Field measurements of CH_4 flux at Loch More

The microtopography of the bog was split according to vegetation type and water table depth into the classes shown in Table 7.1. The field site is shown in Plate 7.1. Peat temperature ranged from 7.8 to 12.5 °C, however, this change was not linear over the measurement period and no significant effect on the CH_4 flux was observed. The flux of CH_4 spanned two orders of magnitude, from a small CH_4 source on the moorland of 6.7 ng m^{-2} s^{-1} to greater than 700 ng m^{-2} s^{-1} from open pool areas vegetated with *Menyanthes trifoliata* (Table 7.1, Figure 7.1). Intra-site CH_4 flux also spanned a wide range, particularly from the hollows where temperatures tended to be larger and ebullition could periodically be observed. In the open water pool areas vascular transport of CH_4 through *Menyanthes trifoliata* was found to be the largest CH_4 source. CH_4 emission averaged 780.4 ng m^{-2} s^{-1} from chambers containing *Menyanthes trifoliata*, compared to 64.4 ng m^{-2} s^{-1} from open water.

Plate 7.1. The Flow Country near Loch More Caithness. The variations in microtopography can be seen with the open pool areas containing the vascular plant *Menyanthes trifoliata* surrounded by lawn and hummock areas.

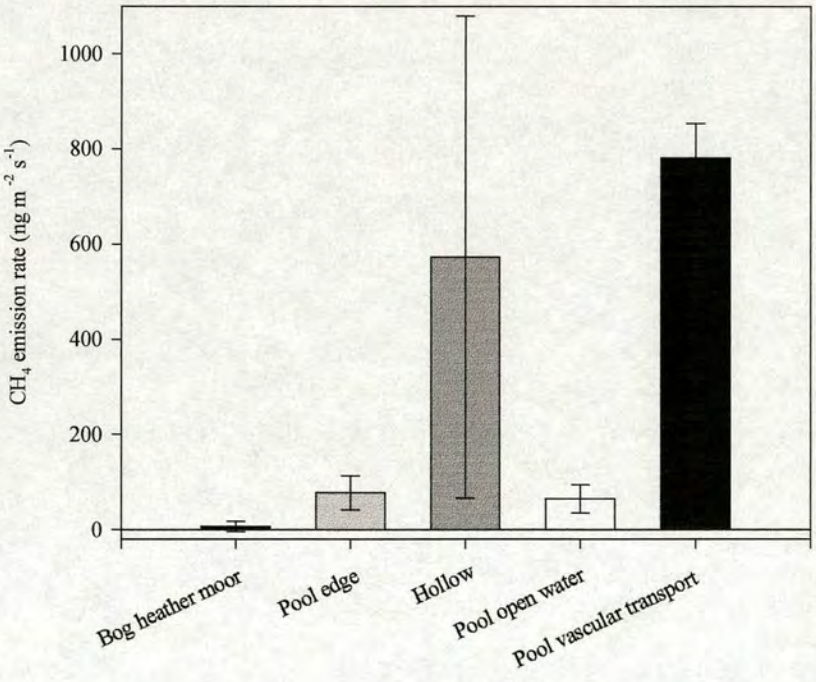

Figure 7.1 Rates of CH_4 emission from the sites studied at Loch More, Caithness. The error bars represent the standard deviation from the mean.

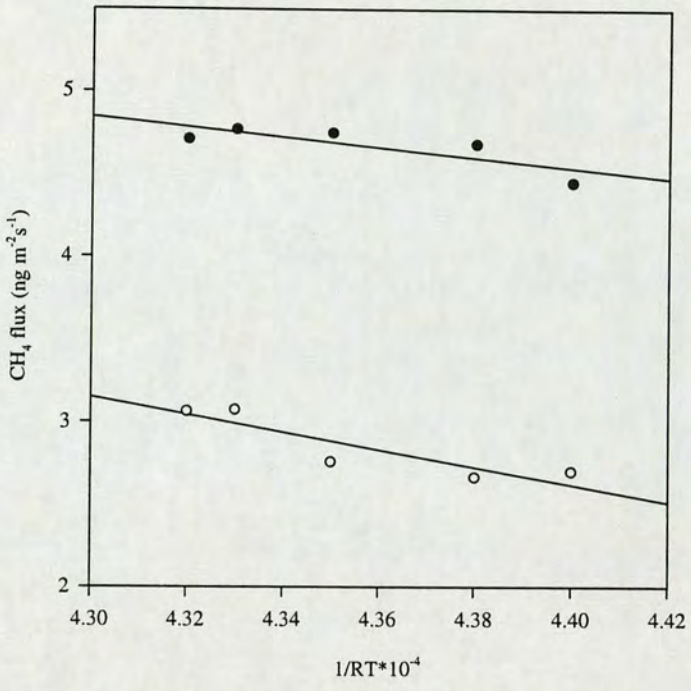

Figure 7.2 Arrhenius plot of the temperature response of CH_4 emission at the (●) *Eriophorum* and (○) *Nardus* sites at the Mine Road, measured during the winter sampling period, March 1995.

7.4.2 Methane flux at Great Dun Fell

Methane flux covered a wide range during the winter measurement period, ranging from a net CH_4 oxidation of -2.1 ng m^{-2} s^{-1} at the Fell Gate grass site to a net emission of 107.4 ng m^{-2} s^{-1} at the Mine Road *Eriophorum* site (Table 7.3). The balance between CH_4 oxidation and emission between the sites investigated was dependent on the water content of the soil. A significant correlation between water content and mean CH_4 flux was observed (r^2 = 0.991, $p<0.05$) with net CH_4 emission being observed at water contents greater than approximately 110 % dry weight.

A significant temperature response was observed at the Mine Road over the winter measurement period, despite only a 5 °C variation in soil temperature. An Arrhenius plot (Figure 7.2) shows an Ea of 56.1 kJ mol^{-1} at the *Nardus* site, compared to 34.8 kJ mol^{-1} at the *Eriophorum* site (equivalent to Q_{10}'s of 2.3 and 1.7 respectively). No other correlations were observed between the environmental variables measured and CH_4 flux rates over the measurement period.

During the summer months flux measurements were made from a greater number of sites. CH_4 flux again covered a wide range, from -5.5 ng m^{-2} s^{-1} to 260.2 ng m^{-2} s^{-1} (Table 7.3). No significant relationships were observed between the mean CH_4 flux at each site

over the sampling period and the environmental variables measured; however, correlations were observed within individual sampling days. Soil water content was the main factor determining CH_4 flux. However, four sites - Fell Gate *Carex* pool, Fell Gate *Carex/Sphagnum*, Mine Road hill *Nardus* and Summit *Carex/Nardus* - did not show any response to the increasing water content of the peat. When these sites were not included in the regression the relationship between CH_4 flux and soil water content was significant ($p<0.05$) on four out of the five measurement days. No other significant correlations were observed over the measurement period, between mean CH_4 flux and the environmental variables measured at the sites studied.

At the Fell Gate sites a significant correlation ($p<0.05$, $r^2=0.676$) between CH_4 flux and soil water content was observed. CH_4 flux ranged from a net oxidation of -5.5 ng m^{-2} s^{-1} at the Fell Gate *Juncus* site to a net emission of 126.7 ng m^{-2} s^{-1} at the Fell Gate mud pool site. CH_4 oxidation rates showed a small increase between the winter and summer sampling periods at the Fell gate *Carex* and Grass sites, concomitant with a reduction in soil water content and an increase in soil temperature.

At the Mine Road sites no significant correlations were observed between CH_4 flux and the environmental variables measured. A significantly larger CH_4 emission was observed from the *Juncus* site on the hill than from the *Nardus* site on the hill, despite similar soil water contents, suggesting that *Juncus* may provide a conduit for CH_4. The adjacent Mine Road *Nardus* and *Eriophorum* sites showed very different CH_4 emission rates, with the flux from the *Eriophorum* site being five times larger in winter and three times larger in summer than the *Nardus* site. The difference was coincident with a large difference in soil water contents (Table 7.3) and vegetation cover. A comparison of the winter and summer CH_4 fluxes at these sites showed that at the *Nardus* site the fluxes were very similar, whereas there was a 25% increase at the *Eriophorum* site in the summer. Soil temperature increased by 8.6 °C between the two sampling periods and soil water content decreased by 23 and 31% at the *Nardus* and *Eriophorum* sites, respectively. At the Mine Road hill *Nardus* site a small net winter sink became a small summer source of 3.8 ng m^{-2} s^{-1}, in association with a doubling of soil water content, probably a result of spatial rather than seasonal variability.

Table 7.3 CH_4 flux, soil water content and temperature at Great Dun Fell, winter and summer field campaigns 1995

	WINTER (March)				SUMMER (July)			
Site	Mean CH_4 flux (std. dev.) (ng m^{-2} s^{-1})	n	Soil temperature (°C)	Soil water content (% dry weight)	Mean CH_4 flux (std. dev.) (ng m^{-2} s^{-1})	n	Soil temperature (°C)	Soil water content (% dry weight)
Fell Gate grass	-2.1 (1.9)	2×5	4.7	52.0	-3.0 (2.6)	3×5	13.1	32.1
Fell Gate *carex*	0.2 (0.6)	1×5		135.0	-2.0 (1.5)	3×5		61
Fell Gate *carex* pool	-	-		-	8.8 (22.0)	1×5		573.1
Fell Gate *carex/sphagnum*	-	-		-	18.1 (36.1)	1×4		542.2
Fell Gate *sphagnum* pool	-	-		-	41.2 (28.0)	1×3		338.7
Fell Gate mud pool	-	-		-	126.7 (102.4	1×3		920.0
Fell Gate *juncus*	-	-		-	-5.5 (0.7)	1×4		97.1
Mine Road hill *nardus*	-0.8 (1.3)	1×4	3.4	118.0	3.8 (3.1)	2×4	12.0	247.6
Mine Road hill *juncus*	-	-		-	105.2 (139.1)	1×4		196.6
Mine Road *nardus*	18.8 (3.6)	1×3,2×2		227.7	17.7 (17.5)	2×5,1×3		174.0
Mine Road *juncus*	-	-		-	117.5 (68.0)	2×4,1×3		293.5
Mine Road *Eriophorum*	107.4 (17.1)	2×5		744.3	120.3 (56.6)	3×5		509.7
Summit grass	-1.35 (1.8)	3×5	1.4	86.7	-3.7 (3.2)	3×5	11.5	71.5
Summit *carex/nardus*	-	-		-	18.5 (38.9)	1×5		334.8
Summit *carex* pool	-	-		-	260.2 (138.9)	1×5		317.8
Summit *carex sphagnum*	-	-		-	214.4 (74.6)	1×5		561.1
Summit *sphagnum* pool	-	-		-	159.8 (263.8)	1×5		surface

n = number of chambers × number of measurement days.

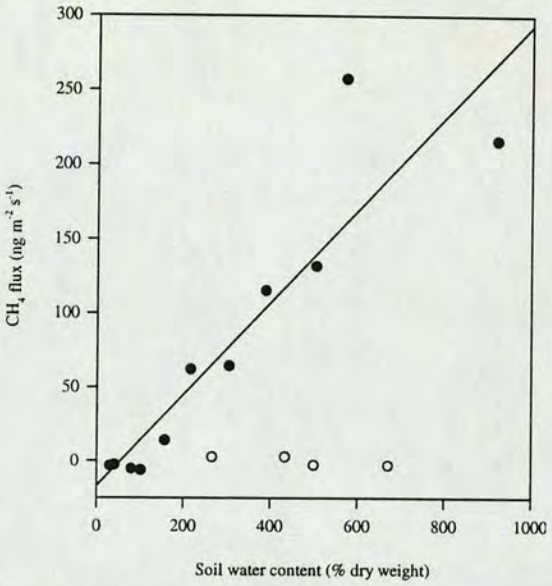

Figure 7.3 The relationship between CH_4 flux and soil water content at all sites during the summer sampling period, July 1995. The regression equation was CH_4 flux (ng m^{-2} s^{-1})=0.3×soil H_2O (% dry wt.)-17.0 (r^2=0.844 $p<0.05$). Four sites (O) showed no response to water conrent.

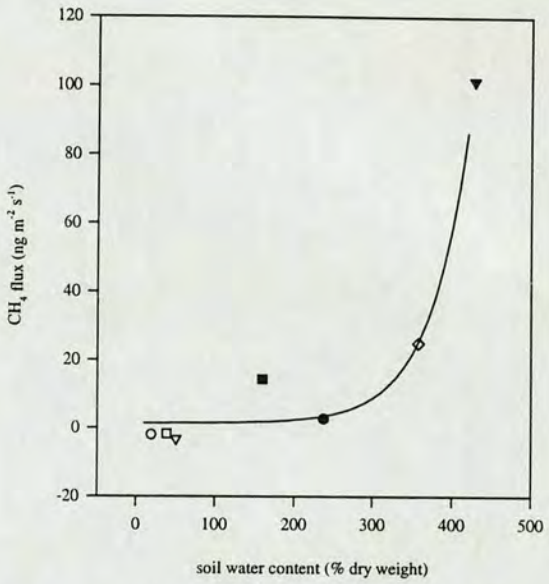

Figure 7.4 The response of CH_4 flux to water content at the 'grass and sedge' sites. The equation describing the relationship was: CH_4 flux (ng m^{-2} s^{-1})=0.02exp(0.02×soil H_2O(% dry wt.)) +1.3 (r^2=0.967 $p<0.001$).

At the summit (Table 7.3) sites CH_4 flux spanned the widest range from -3.7 ng m^{-2} s^{-1} at the grass site, to 260.2 ng m^{-2} s^{-1} at the *Carex* pool site. At the *Sphagnum* pool site where the water table was at the surface, flux variability was high, probably due to ebullition. A comparison between winter and summer flux was only possible at the summit grass site, where the CH_4 oxidation rate more than doubled, coincident with a reduction in soil water content and increase in soil temperature.

In order to examine the relationship between CH_4 flux and vegetation type, flux measurements were split according to the vegetation at each site into the following groups: a) grasses and sedges, b) *Sphagnum* containing sites, c) *Juncus* and d) dry pools. At the grass and sedge sites an exponential relationship between soil water content and CH_4 flux was observed (Figure 7.4). At the *Sphagnum* sites CH_4 flux was over an order of magnitude lower at the Fell Gate site than at the Summit site, probably as a result of the shallow peat layer. CH_4 flux also covered a wide range at the sites dominated by *Juncus*, and both CH_4 emission and oxidation were observed. The dry pool areas covered a wide range of water contents and CH_4 emission rates. Several factors contributed, including temporal variation in soil water content, and intersite variability. No significant relationships between CH_4 flux and soil parameters were observed and no significant differences in soil available NO_3-N and NH_4-N were found between the three altitudes (Table 7.4).

Table 7.4 Soil characteristics at Great Dun Fell.

Site	NO_3-N ($\mu g\ g^{-1}$ dry soil)	NH_4-N ($\mu g\ g^{-1}$ dry soil)	Bulk density ($g\ cm^{-3}$)	pH ($CaCl_2$)
Fell Gate grass	1.3	15.5	0.9	3.1
Fell Gate *carex*	1.0	18.3	0.5	3.6
Fell Gate *carex* pool	0	27.7	0.3	-
Fell Gate *carex/sphagnum*	0	29.2	-	-
Fell Gate *sphagnum* pool	-	-	-	-
Fell Gate mud pool	-	-	-	-
Fell Gate *juncus*	1.2	9.74	0.6	5.8
Mine Road hill *nardus*	0	24.0	0.4	-
Mine Road hill *juncus*	0	14.3	0.5	-
Mine Road *nardus*	0	12.0	0.6	4.1
Mine Road *juncus*	0	15.8	0.2	-
Mine Road *Eriophorum*	0	24.0	0.3	4.1
Summit grass	0	8.5	0.8	3.4
Summit *carex/nardus*	0	20.6	0.7	3.3
Summit *carex* pool	0	8.3	0.3	-
Summit *carex sphagnum*	0	15.4	-	4.3
Summit *sphagnum* pool	-	-	-	4.3

7.4.3 Temperature response investigation

The rate of CH_4 emission from each core in the CONVIRON increased exponentially with increasing temperature between 5 and 30 °C. The response from Core 2 is shown in Figure 7.5a. Arrhenius plots (Figure 7.5b) gave activation energies ranging between 51.8 and 73.2 kJ mol^{-1} and Q_{10} values were between 2.2 and 3.0 (Table 7.5). Following the temperature response investigation the monoliths were maintained at 15 °C, however, despite an initial decrease (in monoliths 1 and 2 only), following the temperature drop from 30 to 15 °C, the CH_4 emission continued to increase (Figure 7.6). The rate of CH_4 emission from monolith 2 was greater after 9 days at 15 °C (~1150 ng $m^{-2}\ s^{-1}$), than it had been at 30 °C (~700 ng $m^{-2}\ s^{-1}$).

7.4.4 Effects of sulphate application

The application of Na_2SO_4 to pool monoliths in the OTC reduced the CH_4 emission below that of the control monolith (Figure 7.7). The 3 measurements before the treatment were meaned and compared to the last 3 measurements after treatment. The S treated core had decreased by 41% compared to a 25% decrease in the control core. The first measurement,

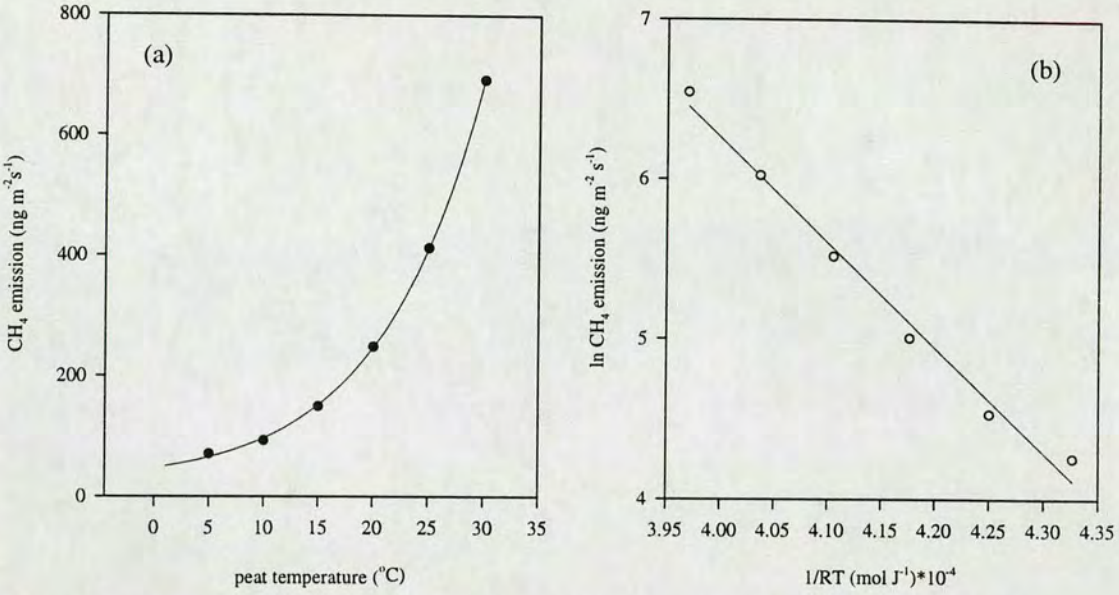

Figure 7.5 a) The temperature response and, **b)** Arrhenius plot, for CH_4 emission from peat monolith, 2. The regression equation describing the relationship was CH_4 flux = 24.97*exp(0.11*temp.)+22.42 and the activation energy was 65.5 kJ mol^{-1}.

Table 7.5. The temperature response characteristics of the peat monoliths

Pool core	mean CH_4 emission (15 °C) (ng m^{-2} s^{-1})	Activation energy (kJ mol^{-1})	Q_{10}
1	402.4	73.2	3.0
2	151.8	65.5	2.7
3	294.3	51.8	2.2

made only a few hours after the application of S, produced a large peak in CH_4 emission which was approximately double the mean emission throughout the measurement period from this monolith. The large variability in temperature in the made the response difficult to clearly identify. The results from the second S application experiment in the more controlled conditions of the CONVIRON are shown in Figure 7.8. Because CH_4 emission rates were increasing before the S application, curves were fitted, from the first 7 days of measurements, to predict the equilibrium emission rate. The reduction in CH_4 emission was calculated from the percentage reduction between the predicted and actual emissions. Both S treatments showed a significant reduction in CH_4 emission rates with the Na_2SO_4 treatment having a larger effect than the $(NH_4)_2SO_4$ treatment (Table 7.6). A large stimulation of CH_4 emission was again observed immediately following S application.

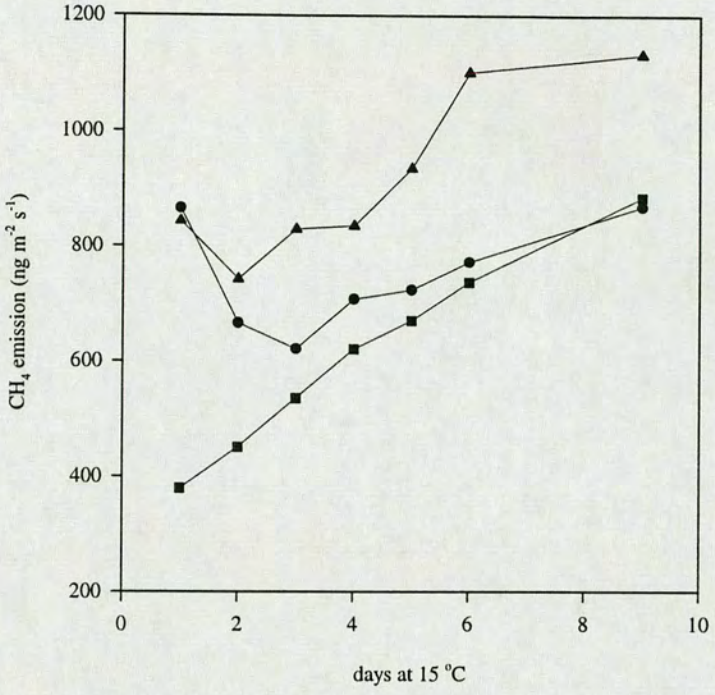

Figure 7.6 Rates of CH_4 emission at 15 °C from the three peat monoliths following the temperature response investigation.

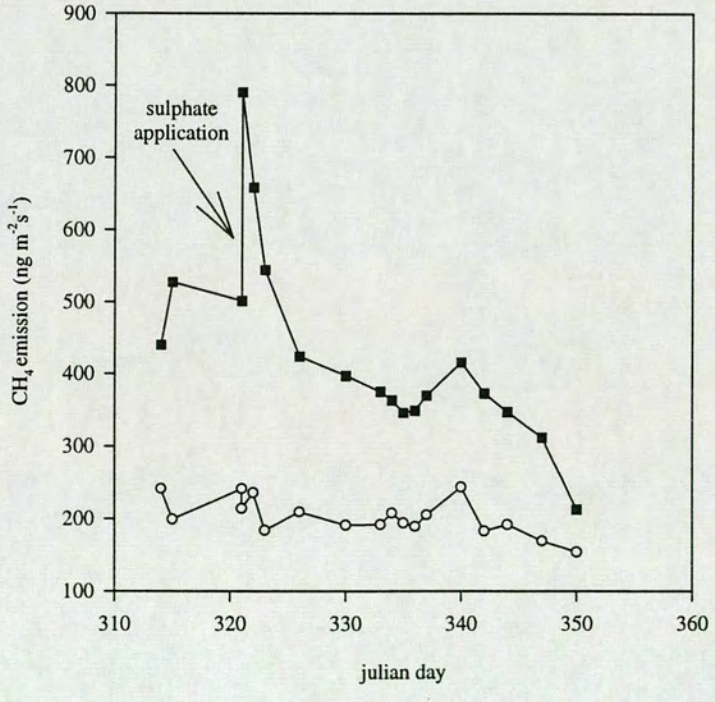

Figure 7.7 The effect of S application (100 kg ha^{-1}) on CH_4 emission rates from a pool type peat monolith in an OTC. (○) control and (■) treated monolith.

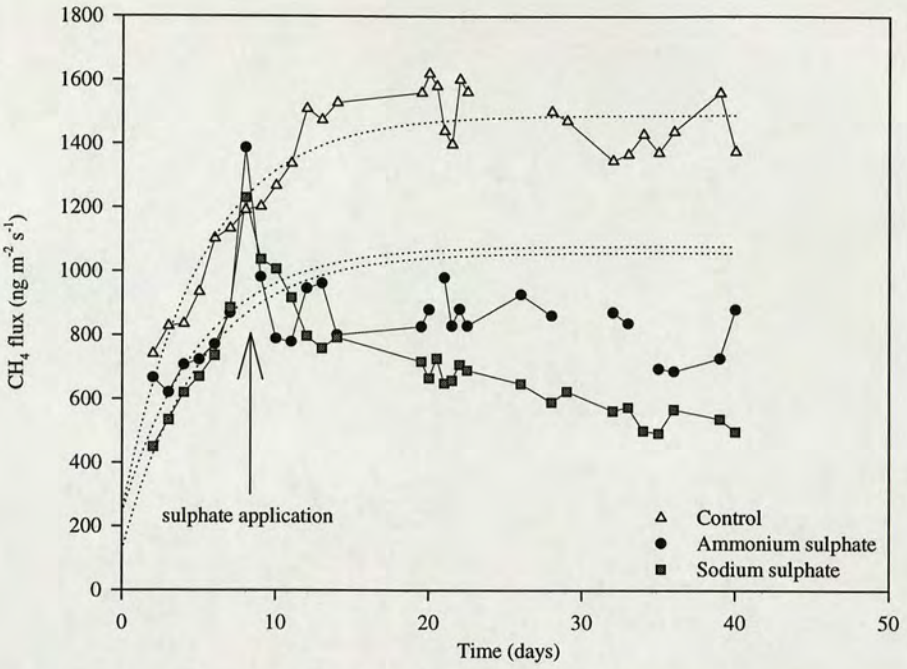

Figure 7.8 The effect of S application at 100 kg ha^{-1} $(NH_4)_2SO_4$ and Na_2SO_4 on CH_4 emission rates from peat monoliths under controlled conditions. The dotted lines show the fitted curves of the predicted CH_4 emission equilibrium rate.

Table 7.6. The relative reduction in CH_4 emission following the application of 100 kg ha^{-1} S.

	Control	$(NH_4)_2SO_4$	Na_2SO_4
Actual CH_4 flux (ng m^{-2} s^{-1})	1436.4	747.5	524
Modelled CH_4 flux (ng m^{-2} s^{-1})	1489.8	1078.7	1057.8
% Reduction	-	30.7	50.5

7.4.5 CH_4 emission through Menyanthes trifoliata

Methane emission was observed to occur through the stems of *Menyanthes trifoliata* in a range of plants in peat monoliths in the OTC. It was found that the emission occurred through the stems of the plants and no detectable flux was observed through the leaves alone. No significant correlation was observed between stomatal conductance and CH_4 emission rates, although the range of stomatal conductances was low. Peat temperature affected CH_4 emission, with significantly ($p<0.05$) larger fluxes at 20 °C than at 10 °C. CH_4 fluxes ranged from 3.5 to 1211 ng m^{-2} s^{-1}. The internal structure of the *Menyanthes trifoliata* stem is shown in Plate 7.2.

7.5 Discussion

7.5.1 Field measurements of CH_4 flux at Loch More

The wide range of CH_4 emission rates observed in this study is typical of most wetland environments (Crill *et al.*, 1988, Moore *et al.*, 1990, Dise *et al.*, 1993). Variations in the microtopography, on a scale of less than 1 m^2, leads to small scale spatial changes in parameters, such as redox potential, water table depth, presence or absence of vascular plants, which strongly affect CH_4 production. Water table depth and plant transport appeared to be the most important factors affecting the CH_4 flux from the range of sites studied with the largest flux being observed through the vascular plant *Menyanthes trifoliata* in the pool areas. However, temperature would presumably also have been important had Measurements been made over a longer time period. Small rates of CH_4 emission and oxidation were observed from moorland areas where the water table was >100 cm below the surface, whereas rates of CH_4 emission were two orders of magnitude larger, when the water table was at or above the surface of the bog. Water table depth has generally been found to be the major controlling factor affecting CH_4 emission rates. Dise *et al.* (1993) observed that water table position controlled 62% of the variance from a range of peatland ecosystems in northern Minnesota, and Moore & Dalva (1993) observed strong relationships between water table height and CH_4 emission in peat cores. CH_4 flux along a soil water content gradient is represented schematically in Figure 7.9.

7.5.2 Transport of CH_4 through plants

The largest source of CH_4 observed in the sites studied at Loch More, was through *Menyanthes trifoliata.* CH_4 flux increased by an order of magnitude when flux measurements included plants as opposed to just open water. The transport of CH_4 through vascular plants has been subject to several recent investigations, and it has been estimated that plant dependent emission can account for up to 90% of CH_4 flux from wetlands and paddy fields (Whiting & Chanton, 1992, Yavitt & Knapp, 1995, Shannon *et al.*, 1996).

Many wetland plants have adapted to anoxic conditions by developing large intercellular spaces and arenchyma which allow the movement of oxygen into the roots (Armstrong, 1972), and also allow the diffussion of CH_4 from anoxic layers to the atmosphere (Sebacher *et al.*, 1985). Plate 1 shows the aerenchyma present in the stem of *Menyanthes trifoliata* for gaseous exchange. Wetland plants also affect the net CH_4 flux by providing root exudates which stimulate CH_4 production (Schηtz, 1991, and references therein), and by the transport of oxygen into the rhizosphere that supports a methanotrophic

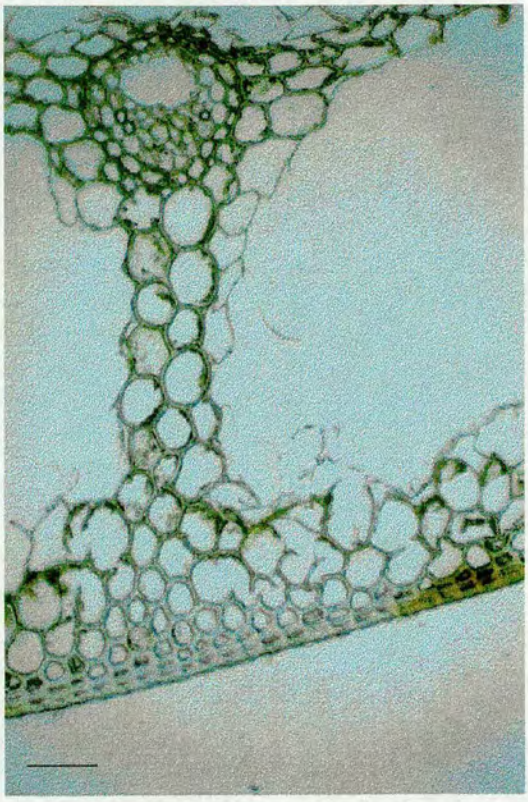

Plate 7.2. Aerenchyma present in the stem of *Menyanthes trifoliata*. Scale bar = 75 μm.

community, which has been estimated to oxidise up to 80% of the CH_4 produced (Holzapfel-Pschorn *et al.*, 1985, Schipper & Reddy, 1996). The transport of CH_4 from soil to the plant has been investigated in several studies. Conrad (1993) suggested that gas bubbles in contact with the roots of rice plants may facilitate the diffusion of CH_4 from the soil to the plants aerenchyma. Nouchi *et al.* (1993) suggested that dissolved CH_4 in soil water surrounding the roots diffused into the cell wall water of the root cortex, driven by the concentration gradient, where it was gasified and transported to the shoots through aerenchyma and intercellular spaces. Investigations into the control of CH_4 emission from the plant to the atmosphere show inconsistent results. Stomatal control has been observed to be important only in a few studies, with clear diurnal patterns in CH_4 emission being observed (Yavitt & Knapp, 1995, Thomas *et al.*, 1996). Morrissey *et al.* (1993) observed a positive correlation between CH_4 emission and stomatal conductance but significant CH_4 flux was observed even when stomata were closed. Many studies have found no stomatal control on CH_4 emission rates (Seiler *et al.*, 1984, Holzapfel-Pschorn *et al.*, 1986, Whiting & Chanton, 1996) and Nouchi *et al.* (1993) found that the main source of CH_4 emission from rice plants was from micropores located on the abaxial epidermis of the leaf sheath.

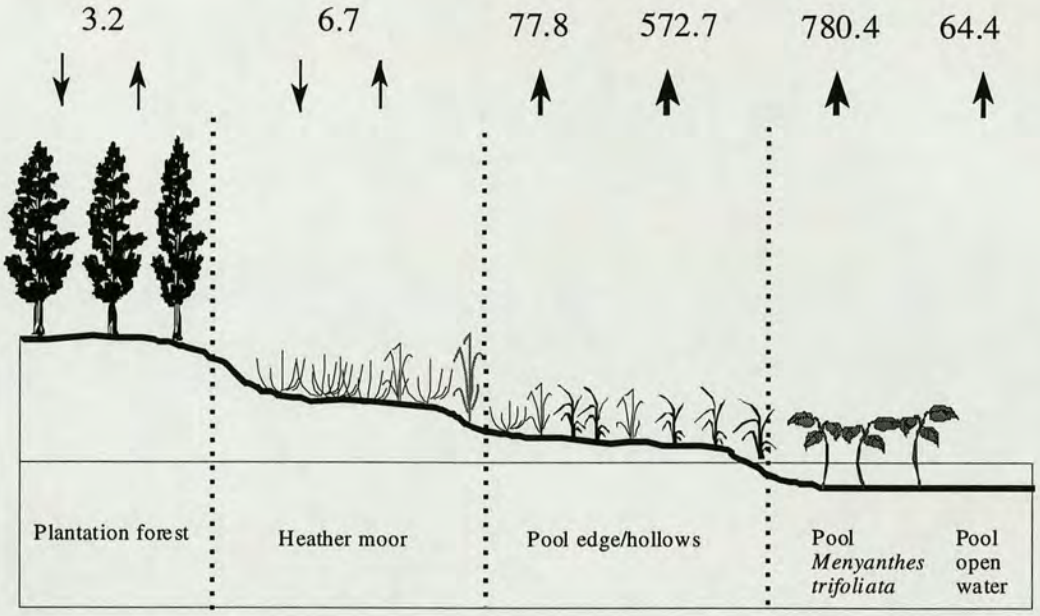

Figure 7.9 Summary of the CH_4 flux data from the sites studied at Loch More. The net flux to the atmosphere is shown above the arrows which represent CH_4 emission↑ or oxidation ↓. Although net emission was observed from all the sites, rates of CH_4 oxidation were observed within some sites. The data from the plantation forest are from MacDonald *et al* (1996) and are discussed in Chapter 3.

In this study presence or absence of light had no effect on CH_4 emission. However, the discovery that most of the CH_4 was escaping from the stem before it reached the leaves implies that it is unlikely that stomatal control would have any effect. An examination of leaves and stems showed virtually no stomata on the stem compared to the leaves. The mode of CH_4 releases from these stems is unknown, it is possible that micropores exist as observed by Nouchi *et al.* (1993). *Menyanthes trifoliata* are widespread throughout the peatlands of Caithness and Sutherland (Ratcliffe and Oswald, 1988) and will play a significant role in the net CH_4 flux. A large degree of seasonality due to the growth stages of the plant is likely, as is seen in rice paddies (Holzapfel-Pschorn *et al.*, 1985). During the winter anabiosis occurs and in spring when the first shoots appear a large pulse of CH_4 is likely, as a result of the restricted transport of CH_4 over the winter period causing CH_4 concentration in the sediments to build up.

7.5.3 Field measurements of CH_4 flux at Great Dun Fell

At Great Dun Fell CH_4 flux covered a wide range, reflecting the diverse microtopography of the sites. Soil water content was the primary controlling factor affecting variations between sites in CH_4 flux, however, the relationship was complex. Generally, CH_4 oxidation rates decreased and CH_4 emission rates increased with increasing soil water content. The negative

relationship between soil water content and CH_4 oxidation rates is well established (Chapter 5, Castro *et al.*, 1994, Lessard *et al.*, 1994). As the air-filled pore space becomes increasingly saturated oxidation rates will decrease to zero. As soil water content continues to increase, the redox potential of the soil decreases, inorganic hydrogen acceptors are consumed, methanogenic activity increases and populations grow, and as a consequence CH_4 production begins. During both the summer and winter sampling periods the response to soil water content was linear, with the exception of four of the sites studied (summer sampling period) where CH_4 flux did not respond to large soil water contents. Other factors, such as slope, peat aeration, and peat depth must have controlled flux at these sites.

The seasonal variation between the winter and summer sampling periods was quite small. At the sites where net CH_4 oxidation occurred, increased oxidation rates were observed in response to decreasing soil water content and increasing soil temperature, although the rates of oxidation were still very small. At the Mine Road *Nardus* and *Eriophorum* sites the seasonal response was very small. Both these sites showed significant temperature responses within the winter sampling period; however, despite a large temperature increase between the winter and summer measurement periods only a small increase in CH_4 emission at the *Eriophorum* site was observed. The large increase in temperature was accompanied by a reduction in peat water content and water table height, which have been shown in several studies to be the rate-limiting factor (Crill *et al.*, 1988, Dise *et al.*, 1993). Also a large proportion of CH_4 produced deeper in the peat profile may have been oxidised before reaching the atmosphere. The temperature responses during the winter sampling period, with a lower E_a observed at the *Eriophorum* site compared to the *Nardus* site, may have been associated with substrate supply. Conrad *et al.* (1987) observed that E_a was positively correlated with substrate supply. The *Nardus* site may have had a more plentiful supply of substrate due to the greater vegetation cover (100%) than at the cotton grass site (<25%), therefore providing a greater concentration of root exudates, easily decomposable organic substances that have been associated with increased rates of CH_4 production (Schütz *et al.*, 1991).

When the sites were grouped according to vegetation type a significant correlation between soil water content and CH_4 flux was observed at the grass and sedge sites. Dry conditions and hence CH_4 oxidation or low rates of emission were associated where grasses such as *Festuca* spp. were dominant. *Carex* and *Nardus* spp. were associated with a wide range of peat water contents, and *Eriophorum* was associated with the wettest site and hence largest rate of CH_4 emission. The use of vegetation as an indirect indicator of CH_4 flux has

been investigated in several studies (Svensson & Rosswall, 1984, Bartlett *et al.*, 1989). Bubier *et al.* (1995) observed that hydrology was one of the main controls on species distribution, and that water table height was correlated with CH_4 flux in two northern peatlands. However, in this study other factors, such as peat depth, were more important, and some species spanned a wide range of soil water contents; eg. *Carex* spp. was present at the Fell Gate at 39% (dry weight) and also at the summit at 357% (dry weight).

Soil available N concentrations did not affect CH_4 flux. Despite the deposition of NH_4^+ and NO_3^- in cloud and rainwater being more than double at the summit than at 244 m (Fowler *et al.*, 1988), no effect of N deposition was observed on the soil available N concentrations. Indeed, between the two most comparable sites, available NH_4-N was greater at the Fell Gate (at 440 m) than at the summit (847 m). However, the grazing of sheep may be an important additional N source masking N deposition effects. N deposition at altitude has been previously shown to inhibit CH_4 oxidation rates (MacDonald *et al.*, 1997/Chapter 4), however, the effects of N deposition on CH_4 emission are less well known. SO_4^{2-} deposition was also enhanced at the summit relative to 244 m, the inhibitory effects of SO_4^{2-} on CH_4 production are discussed below (7.5.5). The combined effects of the above ions on the production and oxidation of CH_4 are difficult to predict and are in need of further investigation.

Methane flux at Great Dun Fell was at the low end of the range of fluxes observed at Loch More, where CH_4 emission through *Menyanthes trifoliata* was about three times the largest emission rate at GDF. The differences are most likely a result of peat depth and water table height with the thin upland, seasonally anaerobic peat at GDF not supporting such an active methanogenic population.

7.5.4 Temperature response of CH_4 emission

Methane emission rates showed a strong exponential response to increasing temperatures between 5 and 30 °C. Q_{10} values and activation energies were in a similar range to other studies (Conrad *et al.*, 1987, Dise *et al.*, 1993). The strong positive relationship between CH_4 production and temperature has been well characterised under laboratory conditions (Williams & Crawford, 1984, Svensson, 1984), with Q_{10}'s between 2.5 and 3.5 and E_a ranging from 60 to 90 kJ mol^{-1} (Conrad, 1989). However under field conditions the relationship is not always clear (Whalen & Reeburgh, 1988, Moore & Knowles, 1990) and can span a wide range of activation energies and Q_{10} values. For example Crill *et al.* (1988) observed E_a values between 116 and 177 kJ mol^{-1} and Q_{10}'s between 5.4 and 13 from

Minnesota peatlands, and Dise *et al.* (1993), also from Minnesota peatlands, observed Q_{10} values between 2.7 and 7.9. Temperature may affect a range of parameters, apart from the metabolic activities of the methanogens themselves, which will alter the net CH_4 flux. For instance, net CH_4 emission may be affected by the different temperature responses of the CH_4 oxidising and CH_4 producing microbial communities (Dunfield *et al.*, 1993), by hysteresis effects due to falling and rising water table (Moore & Dalva, 1993), or by the growth stages of vascular plants. CH_4 production may be affected by the rate of population growth of methanogens, or by the concentration and availability of methanogenic substrates. Because methanogenic bacteria depend on a suite of other microorganisms for provision of substrates, a further range of temperature responses are involved. Conrad *et al.* (1987) showed that temperature limited hydrogen turnover, and hence availability of substrate, to a greater extent than methanogenic activity, and that E_a's were larger in the presence of substrate additions. Svensson (1984) observed the presence of two methanogenic populations with different temperature optima, an acetogenic population with an optimum of 20 °C, and a hydrogen oxidising population with an optimum of 28 °C. Therefore Q_{10} and E_a values may not just be representative of the process under investigation, but may be any combination of the above, which could explain the large variability in these parameters observed in the field.

The continuing increase in CH_4 emission rates, after the temperature response investigation had been carried out and the cores were returned to 15 °C, demonstrates the role of factors other than metabolic effects influencing CH_4 emission rates. Microbial populations may have continued to increase in response to increased substrate supply, as a result of the effect of temperature on decomposition and mineralisation processes. Higher temperatures would almost certainly have stimulated plant growth, resulting in the increased production of root exudates. These easily decomposable substances are known to stimulate CH_4 production (Conrad, 1989). However, it should be noted that long term increases in temperature may have a different result, as the balance between substrates and microbial populations reaches equilibrium and possible changes in vegetation cover take place, and therefore care should be taken when using the results of short-term laboratory experiments to predict the effects of likely changes in climate.

Methane emission rates from the peat monoliths were similar to those observed from static chamber measurements in the field (Table 7.1) and from micrometeorological methods over blanket bog in Caithness (100-200 ng m^{-2} s^{-1}) (Fowler *et al.*, 1996), and well

within the extremely large range of -9.2 to 11200 ng m^{-2} s^{-1}, reported for northern wetlands in a review by Bartlett & Harriss (1993).

7.5.5 Effect of sulphate application

Inputs of sulphur significantly inhibited, by up to 50%, the net flux of CH_4 to the atmosphere. A similar inhibition has been observed in several studies (Abram & Nedwell, 1978, Westermann & Ahring, 1987, Nedwell & Watson, 1995). By acting as an alternate electron acceptor, the presence of SO_4^{2-} allows SO_4^{2-}-reducing bacteria to outcompete methanogens for common substrates, such as H_2 and acetate (Kristjansson *et al.*, 1982). The competitive inhibition of methanogenesis by SO_4^{2-} reducing bacteria is a consequence of thermodynamic and kinetic differences between the two processes (Lovely *et al.*, 1982, Schoheit *et al.*, 1982). Despite the large SO_4^{2-} application rate of 100 kg ha^{-1} (equivalent to approximately twice the annual input to the peatlands in the Pennine Hills (Fowler *et al.*, 1995), CH_4 emission rates were still significant after 40 days, probably due to the presence of non-competitive substrates such as methanol and trimethylamine (Oremland & Polcin, 1982), which can allow methanogenesis and SO_4^{2-} reduction to occur simultaneously (Oremland, 1988). Recent work (Fowler *et al.*, in prep.) has shown that more realistic (lower) concentrations of S also resulted in a significant inhibition (~20%) of CH_4 emission rates. The inhibitory effect of Na_2SO_4 was larger than that of $(NH_4)_2SO_4$, possibly due to inhibitory effects of NH_4-N on methanotrophs, which could partly counteract the inhibitory affect on methanogens, although NaCl has also been observed to adversely affect CH_4 oxidation rates (MacDonald *et al.*, 1997, Chapter 4). NH_4Cl and NaCl additions have been observed to stimulate CH_4 emission from peat by up to 74% and 16% respectively, (Fowler *et al.*, in prep.). Wetlands are often N-limited and the addition of NH_4-N, an essential nutrient for methanogens (Oremland, 1988), may have stimulated methanogenesis. Zeikus & Winfrey (1976) observed a positive correlation between numbers of methanogens and amounts of available NH_4-N in lake sediments. NH_4-N may have stimulated plant growth, an increase in root biomass would increase the potential for CH_4 transport through vascular transport (see 7.5.2). NH_4-N may also have stimulated root exudate production, resulting in increased rates of CH_4 production (Conrad, 1989).

The marked stimulation in CH_4 emission immediately following S application is most likely due to a short term inhibition of methanotrophs; however, they appeared to recover quickly as CH_4 emission returned to pre-S addition levels. This is in contrast to the inhibition of methanotrophs in aerobic soils, which have been observed to have a very long

recovery time > 100 years (Priéme *et al.*, 1997). However substrate supply may be linked to recovery time (King & Schnell, 1994) and in wetland environments obviously would not be limiting, thereby promoting immediate recovery.

The net effect of the various components of acid deposition, such as HNO_3^-, NO_2 and NH_4^+, is in need of quantification. Counter-effects between the various deposited ions may be found, as well as their relative effects on both methanogenesis and methanotrophy. NO_3-N addition to rice paddies has been observed to stimulate CH_4 emission (Lindau *et al.*, 1991), while some N compounds have been observed to inhibit methanogenesis (Balderston & Payne, 1976, Knowles, 1976, Fowler *et al.*, 1994). The mode of application also appears to be important. Small, regular doses of S have been observed to result in a greater degree of inhibition than was observed in this study (N. Dise pers. comm.).

7.6 Summary

Methane flux covered a wide range, from an emission of 780.4 ng m^{-2} s^{-1} through the vascular plant *Menyanthes trifoliata* in a pool area of blanket bog in NE Scotland, to a net oxidation of -5.5 ng m^{-2} s^{-1} from an upland moorland in NW England. Soil water content and vascular transport were important factors determining the magnitude of the flux in the field. Controlled investigations showed that CH_4 emission rates were very responsive to changes in temperature (surface water table) with Ea's between 50 and 70 kJ mol^{-1}. An investigation into the transport of CH_4 through *Menyanthes trifoliata* showed that the majority of CH_4 was emitted through the stem rather than the leaves, and that stomatal conductance had no effect on the flux. S deposition (100 kg ha^{-1}) inhibited CH_4 emission rates by up to 50%.

Chapter Eight

Methane emission by termites and oxidation by soils, along a forest disturbance gradient in the Mbalmayo Forest Reserve, Cameroon.

(A version of this chapter has been published in Global Change Biology 4, 409-418, 1998, with P. Eggleton, D.E. Bignell, F. Forzi and D. Fowler as co-authors)

8.1. Abstract

Methane fluxes were measured, using static chambers, along a disturbance gradient in a West African semi-deciduous humid forest. Soil-feeding termite biomass was simultaneously determined, in an attempt to examine its influence on the net soil-atmosphere exchange of CH_4. CH_4 emission rates from individual termite species were determined under laboratory conditions, permitting the gross production of CH_4 to be compared with net fluxes to the atmosphere. Both net CH_4 oxidation (-) and emission were observed, and CH_4 fluxes ranged from -24.6 to 40.7 ng m^{-2} s^{-1}. A statistically significant relationship between termite biomass and CH_4 flux was observed across the forested sites. Rates of CH_4 oxidation were on average about 60% smaller at the clearfelled and *Terminalia* plantation sites than at the near-primary forest site. Two of the disturbed sites were net CH_4 sources during one of the sampling periods. Disturbance of tropical forests, resulting in a decrease in the CH_4 sink capacity of the soil, may therefore increase the contribution of termite-derived CH_4 to the atmosphere. Measurements from the mounds of the soil-feeding termites *Thoracotermes macrothorax* and *Cubitermes fungifaber* from the old plantation site gave a CH_4 emission of 636 and 53.4 ng s^{-1} $mound^{-1}$, respectively. The forest floor surrounding the mounds was sampled in three concentric bands. Around the mound of *T. macrothorax* the soil was a net source of CH_4, estimated to contribute a further 148 ng s^{-1}. Soil surrounding the mound of *C. fungifaber* was mostly a net sink. The mounds of soil-feeding termites are point sources of CH_4, which at the landscape scale may exceed the general sink capacity of the soil, to an extent dependent on seasonal variations in soil water content and level of disturbance.

8.2. Introduction

The rise in atmospheric CH_4 concentrations in recent decades (IPCC, 1994) has been attributed to sources such as paddy rice cultivation, enteric fermentation, fossil fuel exploitation, and landfill (Cicerone & Oremland, 1988, Khalil & Rasmussen, 1990, IPCC, 1994). The wide range in estimates from many of the sources creates a requirement for accurate measurements and investigations into the interaction between CH_4 sources and sinks. One of the most uncertain components of the source strength budget is the contribution of termite-derived CH_4 and its interaction with the CH_4 oxidation capacity of the soil.

Although the estimated contribution of termites to the global atmospheric CH_4 budget has been revised downwards in recent years (Fraser *et al.*, 1986, Khalil *et al.*, 1990, Martius *et al.* 1993), there are still many uncertainties regarding this calculation (Bignell *et al.*, 1996). Recent studies have shown that there are generally consistent differences between feeding groups, with soil-feeding forms producing more CH_4 under laboratory conditions than wood-feeding and litter-feeding forms (Brauman *et al.*, 1992, Rouland *et al.*, 1993, Bignell *et al.*, 1996). This contradicts assumptions made by, for example, Zimmerman *et al.* (1982), Rasmussen & Khalil (1983) and Collins & Wood (1984), that scaling up to global budgets could be based on the gas emissions and population dynamics of easily sampled wood-feeding species. It appears that the abundance and biomass of soil-feeding termites have been underestimated and that these forms frequently dominate assemblages, especially in African humid forests (Eggleton & Bignell, 1995). Soil-feeding termites are found in epigeal mounds formed from compacted faeces, or are wholly subterranean (Lee & Wood, 1971, Wood & Sands, 1978). Foraging is normally within the top 10 cm of the soil (Eggleton & Bignell, 1995), therefore the potential for CH_4 produced in termite guts to reach the atmosphere will be determined by the oxidation capacity of the soil. If termite-derived CH_4 is making a significant contribution to the global CH_4 budget, the greatest contribution to the total will come from the soil-feeding species, the biomass of which is highest in the forests of West Africa (Eggleton *et al.*, 1996).

Net CH_4 emission to the atmosphere is regulated in many environments by CH_4 oxidation. Up to 80% of CH_4 produced in some wetlands is estimated to be oxidised before reaching the atmosphere (Oremland & Culbertson, 1992) and CH_4 emissions from rice paddies and landfill sites have also been shown to be mediated by CH_4 oxidation (Holzapfel-Pschorn *et al.*, 1985, Boeckx & Van Cleemput, 1995). Therefore it seems likely that net emission by termites will also be determined by the oxidation capacity of the soil or mound

material. The environmental parameters which affect the oxidising capacity of the soil, such as soil water content and land use (Keller *et al.*, 1990, Castro *et al.*, 1995, MacDonald *et al.*, 1996) will thus have a strong influence on the net emission from termites to the atmosphere.

In this chapter measurements of CH_4 flux from tropical forest soils are presented. The relationship between termite-derived CH_4 and CH_4 oxidation in soils, and the effect of disturbance on the relationship between the two processes, is examined for a site in southern Cameroon. Measurements from termite mounds and from isolated worker termites of the same species were also made in order to investigate point sources of CH_4.

8.3. Materials and Methods

8.3.1 Site Description

The study was made during November 1995 in the Mbalmayo Forest Reserve, southern Cameroon. The reserve was established in 1949 and is administered by the Cameroon Ministry of Environments and Forests; it covers about 9000 ha. The reserve contains surviving areas of primary or near-primary forest, extensive secondary forest, forestry plantations of various kinds and experimental farmland established after forest clearance. The forest is classified as moist pre-montane tropical forest (Holdridge *et al.*, 1971).

The climate is sub-equatorial, with two wet seasons. The first rains occur between March and June, the second between mid August and November. The mean annual rainfall total for Mbalmayo is 1513 mm; October is the wettest month (283 mm) and January the driest (24.6 mm). Mean monthly temperatures range between 22.5 °C and 25.0 °C (Holland *et al.*, 1992).

Soils within the study area are classified as Kandiudults derived from schists, typically with loamy sand to sandy loam topsoils overlying clay subsoils. Soils are yellowish brown, moderately drained with weak to moderate structure, are generally stongly acid, leached of bases, with low cation exchange capacities and low nutrient (N, P & K) status (Holland *et al.*, 1992).

Development projects being undertaken in the reserve have imposed a range of disturbance treatments under controlled conditions. Four sites were chosen in order to represent a range of disturbance regimes where termite assemblages and biomass were already characterised (Eggleton *et al.*, 1996):

1. Weeded *Chromolaena* Fallow (WCF). This is a part of the International Institute of Tropical Agriculture (IITA) experimental farm site cleared manually from secondary forest

in 1990. The plot was left fallow but weeded to prevent tree regeneration. The plot developed a dense growth of the alien composite *Chromolaena* spp.

2. Young Plantation (YP). A plantation of *Terminalia ivorensis* established in 1987. The site was cleared manually to leave a canopy cover of about 30%. Planting lines were cleared by chain saw at a separation of about 5 m. The lines were weeded to promote early growth of saplings, and thinned after three years.

3. Old Plantation Forest (OP). A plantation of *Terminalia ivorensis* established in 1949 by similar methods as above.

4. Near-Primary Forest (NP). This plot was subject to selective logging approximately 70 years ago, but retains several large mature trees. Disturbance is very light, confined to local villagers gathering fruits and firewood.

8.3.2. Chamber measurements

Sites were measured twice, over two successive periods of one week, one site per day in week one, repeated in week two. The order of site measurement was determined randomly during each measurement period.

Flux measurements across the disturbance gradient were made using the static chamber method described in Chapter 2. Nine chambers were inserted into the soil randomly along a transect, 24 hours prior to the measurements being taken, at each site. Each chamber was measured in duplicate with an equilibration time of 30 minutes between measurements. The samples were collected into 1 l tedlar bags and air freighted to the UK within two weeks, for analysis by GC. Preliminary experiments in which Tedlar bags filled with 2110 ppb CH_4 standard were air-freighted from the UK to Cameroon and returned after 3 weeks showed the change in CH_4 concentration was less than 15 ppb. Immediately after the CH_4 measurement had been made, soil under each chamber was dug out to a depth of 10 cm and the termites present extracted by hand sorting. They were weighed and preserved in 70% ethanol for later identification and counting at the Natural History Museum, London.

Flux measurements were also made over mounds built by *Thoracotermes macrothorax* and *Cubitermes fungifaber* which were considered typical, in shape and location, of the mounds built by these species throughout the reserve. A single chamber, 50 cm high with an internal volume of 62.5 l, was placed placed over the mound and inserted into the soil. 9 additional chambers, 20 cm high with an internal volume of 25 l, were placed in a T-array on three sides of the mound, with a centre to centre chamber separation of 50 cm, thereby creating three transects away from the mound (Figure 8.1). Fluxes were sampled

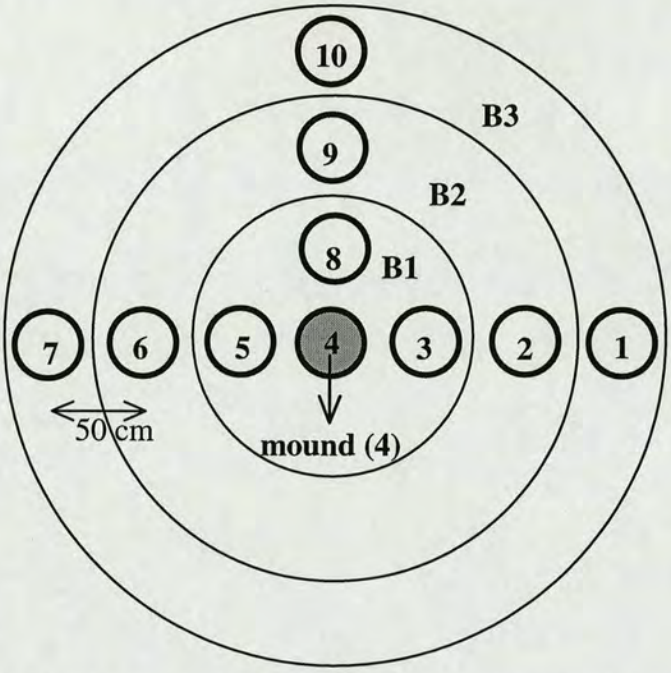

Figure 8.1. Layout of static chambers (40 cm diameter) in a transect array (chambers 1-3 and 5-10 inclusive) away from the mound (chamber 4).

as described above. After sampling, the mounds were removed for dissection and the numbers of termites they contained were determined. Individuals of *C. fungifaber* were counted directly; for *T. macrothorax,* numbers were estimated by a sub-sampling procedure (Eggleton *et al.*, 1996). Soil under each chamber, including the one enclosing the mound, was dug out to a depth of 10 cm and the termites present extracted by hand sorting.

8.3.3. Isolated termite measurements

Measurements of CH_4 emission by isolated termites were made by Dr. D. Bignell (Queen Mary & Westfield College, University of London) using the method described below. Worker termites were brought to the laboratory within 10-20 min of sampling from mounds in the field. They were sorted on moistened filter paper to remove damaged individuals and weighed before being placed in a 100 ml amber gas sampling bottle (Chromatography Services Ltd.), sealed by a Chrompack aluminium crimp cap (20 mm diameter) fitted with a teflon-lined rubber washer. Incubation took place within the laboratory at 26 ± 1 °C. 20 ml of headspace gas were withdrawn with a hypodermic syringe at 1, 2 and 3 hour intervals and transferred to similarly sealed 50 ml Chrompack gas sampling vials from which 20 ml of air had previously been withdrawn by syringe. After each sampling, laboratory air was bled back into the headspace through a syringe needle to equalise pressures. These dilutions were

Plate 8.1. Top - A mound of *Cubitermes heghii* surrounded by static chamber transects as described in 8.3.2. Middle - The soil samples from the pits dug out from underneath the chambers. Bottom - Secondary forest cleared for a pineapple plantation.

factored into the calculation of CH_4 concentrations. CH_4 field standards were established at the same time by injecting 20 ml of a 100 ppm mixture of CH_4 in N_2 into identical vials. Analysis of field standards showed no loss of CH_4 during transit and storage of the vials. Termite mortality was determined at the conclusion of the incubation, and the results from groups where individuals had died during incubation were discarded.

8.3.4. Soil analysis

Soil temperature at 5 cm depth was routinely measured during flux measurements using a portable temperature probe (RS Components, UK). Soil water content was determined at four locations along each site transect during each measurement period by Time Domain Reflectrometry (TDR). Dry bulk density (top 5 cm) was calculated from the mass of oven dry soil in a given volume; three samples were taken from each site transect. Data for organic carbon, total N and soil pH are from Eggleton *et al.* (1996).

8.3.5 Statistical analysis

Relationships between CH_4 flux and termite biomass were analysed using the Pearson product moment correlation and simple linear regression. Significant differences between the measured parameters were determined using an analysis of variance (ANOVA).

8.4. Results and Discussion

8.4.1 CH_4 flux along the forest disturbance gradient

Both CH_4 emission and oxidation were observed; fluxes ranged from -24.6 to 40.7 ng m^{-2} s^{-1}. Oxidation rates were in a similar range to those observed in tropical forest soils in Costa Rica (Keller *et al.*, 1993), Panama (Keller *et al.*, 1990) and central Africa (Tathy *et al.*, 1992). Mean CH_4 fluxes are shown in Table 8.1 and Figure 8.2 from each site on each sampling day. All sites showed a large degree of spatial variability probably as a result of small scale variations in soil permeability, soil moisture and CH_4 production by termites. The largest ($p<0.05$) CH_4 oxidation rates were observed from the relatively undisturbed near-primary forest site, which was a net sink during both sampling weeks. Both the young and old plantations were net sources of CH_4 during the first sampling week but became net sinks during the second measurement week, coinciding with the cessation of rainfall. CH_4 flux measurements were made during the transition period between the wet and dry seasons. During the first sampling week 94.7 mm of rain fell whereas no rain fell during the second sampling period. The weeded *Chromalaena* fallow site, which contained no termites, was

Table 8.1. CH_4 flux measurements and soil characteristics from the forest disturbance gradient

Site	Weeded *Chromolaena* Fallow		Young Plantation		Old Plantation		Near-Primary Forest	
	week 1	week 2	week 1	week 2	week 1	week 2	week 1	week 2
mean CH_4 flux ($ng\ m^{-2}\ s^{-1}$)	-1.9 (1.4)[a]	-7.3 (3.2)	0.3 (7.5)	-10.5 (6.5)	0.6 (15.3)	-3.9 (6.9)	-14.7 (4.9)	-15.2 (10.3)
soil water content ($\%H_2O$)	31.2 (2.1)	25.2 (0.8)	36.4 (1.9)	31.7 (1.8)	30.8 (4.0)	32.2 (1.2)	34.9 (1.6)	29.3 (2.2)
soil temp. (°C)	21.6	23.3	22.2	22.9	21.5	22.3	22.0	22.6
bulk density ($g\ cm^{-3}$)	1.1 (0.2)		1.0 (0.1)		1.0 (0.1)		0.7 (0.1)	
pH ($BaCl_2$)	5.5[b] 4.3		4.2 4.0		nd		3.5 3.5	
organic C (%)	2.5 (0.9)[b] 0.9 (0.3)		2.9 (1.0) 1.4 (0.7)		nd		10.3 (6.7) 3.5 (1.3)	
total N (%)	0.19 (0.03)[b] 0.07 (0.01)		0.20 (0.04) 0.12 (0.04)		nd		0.27 (0.03) 0.20 (0.12)	
C:N ratio	13.3[b] 12.9		14.6 11.6		nd		38.1 17.7	

nd = not determined
[a] mean flux ( ) standard deviation
[b] Upper figure is for top 5 cm, lower figure is 5-50 cm depth

also a net CH_4 sink but at rates between 52 and 88% lower than that of the near-primary forest site. Disturbance in the form of clear felling and/or conversion to plantation resulted in a reduction in the rate of CH_4 oxidation of between 31 and 88 %. The extent of the inhibition varied between sampling weeks, and may have been related to rainfall. The effect of disturbance on CH_4 flux, in the form of forest conversion to agriculture has been observed both in temperate and tropical ecosystems (Keller *et al.*, 1990, Lessard *et al.*, 1994). However, the mechanisms responsible for the observed inhibition are not well understood. The gas transport resistance of a soil is a principal control on CH_4 oxidation rates (Dorr *et*

al., 1993) by limiting the supply of substrate to CH_4 oxidising microorganisms. The observed pattern of CH_4 flux rates could not be explained by differences in soil water content. Soil water content was significantly smaller ($p<0.05$) at the weeded *Chromalaena* and the old secondary plantation sites during the first sampling week than at the other sites, and in the weeded *Chromalaena* site during the second sampling week. Changes in soil structure leading to an increase in soil bulk density and decrease in porosity, that often accompany the conversion of forest to farmland (Reiners *et al.*, 1994), is a likely factor in the inhibition of CH_4 oxidation rates. Soil bulk density was significantly smaller ($p<0.05$), and total organic C was significantly larger ($p<0.05$) at the near-primary site than at any of the disturbed sites, and this may explain the observed differences in CH_4 oxidation rates. Bulk density has been shown to be related to CH_4 oxidation rates across a range of temperate soils (MacDonald *et al.* 1996). A strong inverse relationship between bulk density and organic matter content was observed by Federer *et al.* (1993), and soils with high organic matter content, low bulk density and hence a large degree of pore space (Brady, 1984) allow the rapid diffusion of CH_4 to microorganisms, resulting in potentially large CH_4 oxidation rates.

Methane oxidation rates showed no sign of recovering to pre-disturbance levels, even in the 47-year-old plantation site. The inhibition of CH_4 oxidation rates in the three disturbed sites, relative to the near-primary site was similar, indicating that no recovery in the CH_4 oxidising capacity of the soil had occurred with time. This is in contrast with the findings of Keller & Reiners (1994) who found no significant differences in CH_4 oxidation rates between undisturbed (old growth forest) and disturbed (secondary forest and abandoned pasture) systems in Costa Rica and concluded that soil-atmosphere fluxes of CH_4 were restored to pre-disturbance rates within 50 years. However, in the Keller & Reiners (1994) study soil bulk density also recovered quickly to the pre-disturbance level. Studies in Northern Europe have indicated that recovery of CH_4 oxidation rates to pre-disturbance levels can take > 100 years (Priemé *et al.*, 1997). The reasons for the differences in recovery time are unknown.

The magnitude of the inhibition in CH_4 oxidation rates of the disturbed sites, relative to the near primary site, was greater in the first than in the second sampling week, corresponding with the cessation of rainfall (Fig 8.2). This study was carried out in the transitional period between the wet and dry seasons. During the first week of measurements heavy persistent rain fell every evening but in the second week no significant rainfall was recorded. Soil water content was significantly ($p<0.05$) lower during the second week at all

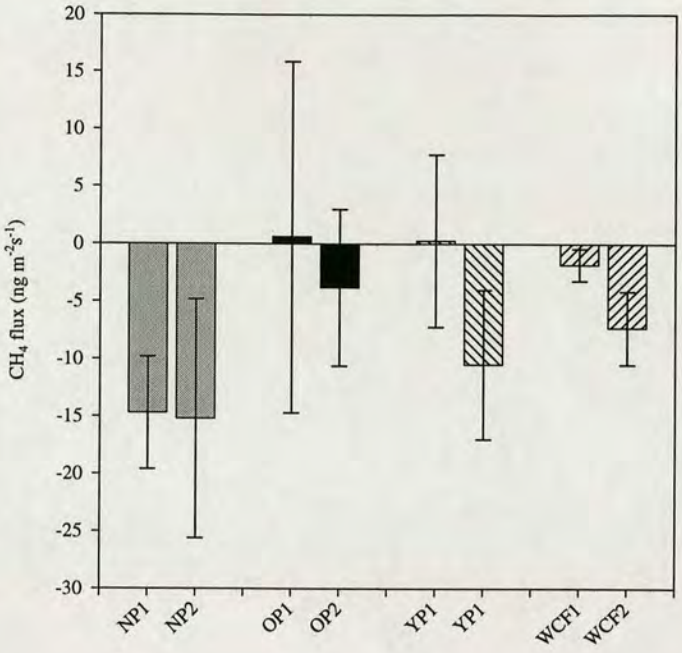

Figure 8.2. Methane flux (± standard deviation) along the forest disturbance gradient; NP near primary, OP old plantation, YP young plantation, and WCF weeded *Chromolaena* fallow in sampling week 1 and sample week 2.

heavy persistent rain fell every evening but in the second week no significant rainfall was recorded. Soil water content was significantly ($p<0.05$) lower during the second week at all sites except the old plantation site. Oxidation rates were significantly larger ($p<0.05$) during the second week at the weeded *Chromalaena* fallow and young plantation sites. Soil water content may take longer to respond at the near-primary and old secondary sites, due to a greater canopy density resulting in a greater rainfall interception capacity. Soil water content has been shown to have a major controlling effect on CH_4 oxidation rates and precipitation events can cause short-term changes in oxidation capacity (Castro *et al.*, 1994). The seasonal change in soil water content accompanying the rainfall pattern of this area may result in a strong seasonal variation in CH_4 oxidation rates. The relatively low oxidation rates observed in this study were representative of the wettest part of the year, hence annual oxidation rates are likely to be higher. Long-term measurements of flux rates are required to quantify the magnitude of the seasonal variation.

Methane oxidation rates have also been shown to be sensitive to inputs of N (Steudler *et al.*, 1989), and therefore altered rates of N cycling following forest clearance (Reiners *et al.*, 1994) may also have a detrimental effect. Total N was largest in the near-primary plot (Table 8.1). However, in contrast to N manipulation studies, where inputs of N have been shown to inhibit CH_4 oxidation rates (Mosier *et al.*, 1991, Sitaula *et al.*, 1995),

Priemθ *et al.*, 1997). Soil pH also showed a gradient across the plot with the highest pH observed in the farm fallow and the most acidic soil being the near primary forest site. The effect of soil pH on CH_4 oxidation rates is uncertain with reports of acidity both increasing (Sitaula *et al.*, 1995) and decreasing oxidation rates (Hητsch *et al.*, 1994).

Termite biomass showed a significant correlation with CH_4 flux across the forested sites (r^2=0.268, $p<0.05$). The weeded *Chromalaena* fallow site was not included in the regression because it contained no termites. Six chambers showed a net emission of CH_4, with fluxes ranging from 0.3 to 40.8 ng m^{-2} s^{-1}. Small rates of CH_4 emission were also observed from chambers containing no termites, indicating either that methanogenesis was occurring in the wet soil, possibly in anaerobic microsites, or that the CH_4 observed was diffusing through adjacent termite galleries. More sampling is required to cover the mid-range of termite biomass; however, marked clumping in termite densities (Eggleton *et al.*, 1996) makes intermediate and high biomasses uncommon. The potential for methanotrophs to oxidise CH_4 produced by soil feeding termites is regulated by the environmental conditions that affect methanotrophs in soil, such as soil texture, soil water content and level of disturbance (Dorr *et al.*, 1993, Keller *et al.*, 1993, Castro *et al.*, 1994). Any disturbance which reduces the sink strength of the soil may increase the amount of termite-derived CH_4 escaping to the atmosphere. In this study only the disturbed sites were net sources of CH_4 to the atmosphere. This emission appeared to be dependent on soil water content and precipitation, indicating a likely seasonal dependence of CH_4 flux. It has been recently observed (Dibog *et al.*, unpublished results) that termite biomass also has a pronounced seasonality, therefore the balance between CH_4 production by termites and CH_4 oxidation by soils may show a complex seasonal variation.

The influence of termites on soil structure and porosity (Brussard & Juma, 1996) could enhance the activity of CH_4-oxidising microorganisms. It is also possible that the CH_4 produced by termites supports a larger and more active methanotroph population. Soil can show an increase in CH_4 oxidising activity, and numbers of methanotrophs, when incubated at greater than atmospheric mixing ratios (Bender & Conrad, 1995).

8.4.2. CH_4 flux from termite mounds

Both mounds were large sources of CH_4 (Table 8.2). The larger mound of *T. macrothorax* produced nearly 12 times as much CH_4 as the *C. fungifaber* mound, averaging 5051 ng $m^{-2}s^{-1}$ (636 ng s^{-1} $mound^{-1}$) and 426.9 ng m^{-2} s^{-1} (53.4 ng s^{-1} $mound^{-1}$) respectively. CH_4 emission from the mound of *T. macrothorax* was about 24% less than predicted from the CH_4

production of termites in the laboratory (Table 8.2). CH_4 oxidation by mound material may explain the lower than expected flux. Khalil *et al.* (1990) calculated that about 40% of the CH_4 produced in mounds was oxidised before reaching the atmosphere. However, the *C. fungifaber* emission was slightly larger (6%) than predicted. The discrepancy between predicted and actual emissions may in part be due to observations that termites isolated from the mound or colony may produce less CH_4 than *in situ*, or may show reduced output when not in contact with soil or mound material (Bignell *et al.*, 1996).

Of the nine transect chambers placed adjacent to the mound of *T. macrothorax*, seven showed a net emission of CH_4, ranging from 4.3 to 56.3 ng m^{-2} s^{-1} (Table 8.2). By contrast, only one of nine chambers placed adjacent to the mound of *C. fungifaber* was a net source of CH_4 (35.6 ng m^{-2} s^{-1}); the other eight chambers showed CH_4 oxidation rates ranging between -0.2 and-14.8 ng m^{-2} s^{-1}. The sampling in the T-array was used to scale up termite abundance, biomass and the net CH_4 flux from the soil in the mound circle of *T. macrothorax* and in the three concentric circular bands to a radial distance of 1.75 m (Table 8.3). For *C. fungifaber* a similar calculation was made, but was confined to the mound circle and the first band (to a radial distance of 0.75 m), as only chambers in this area were found to have termites in the underlying soil (Table 8.3). The *T. macrothorax* mound was a very large point source, with the area around the mound contributing a further 148 ng m^{-2} s^{-1}. There was some variation in the source strength around the mound, with band 2 being the largest source despite band 1 having the largest termite biomass, probably due to foraging tunnels radiating out from the centre of the nest which act as conduits for CH_4. With the much smaller *C. fungifaber* mound, all three concentric bands were net sinks. Comparisons with Delmas *et al.* (1992), who also give emissions relative to the enclosed area, show a major difference with an emission of only 100 ng m^{-2} s^{-1} reported for *T. macrothorax*. They also measured a flux of 2710 ng m^{-2} s^{-1} for an unidentified species of *Cubitermes,* compared with the emission of 427 ng m^{-2} s^{-1} reported in the present study for *C. fungifaber*. However *C. fungifaber* has one of the smallest mounds of any species in the genus. Seiler *et al.* (1984) gave the absolute flux of CH_4 from a *Cubitermes* mound in southern Africa as 19.4 ng s^{-1}, but the area was not specified. CH_4 emission from termite mounds vary widely according to species composition and biomass which makes scaling up difficult.

Table 8.2 Measured CH_4 fluxes and predicted CH_4 fluxes (scaled up from isolated termite measurements of CH_4 production) from the mounds and transects of *T. macrothorax* and *C. fungifaber*

Chamber	Mean CH_4 flux (ng m^{-2} s^{-1})	Termite abundance (workers $sample^{-1}$)	Termite biomass (g m^{-2})	Predicted CH_4 flux (ng m^{-2} s^{-1})	Predicted/actual flux * 100 (%)
A. *Thoracotermes macrothorax*					
1	-5.9	0	0	0	0
2	-4.7	29	2.61	2.8	-59.6
3	21.2	620	56.6	60.7	286
4 (mound)	5050	64000	5840	6260	124
5	27.9	20	1.8	2.0	7.2
6	56.3	13	1.2	1.3	2.3
7	4.3	3	0.2	0.3	6.3
8	7.2	75	6.8	7.3	101
9	49.1	35	3.2	3.4	6.9
10	16.1	1	0.1	0.1	0.6
B. *Cubitermes fungifaber*					
1	-9.9	0	0	0	0
2	-8.7	0	0	0	0
3	-0.2	0	0	0	0
4 (mound)	427	5500	346	404	94
5	-8.9	0	0	0	0
6	-14.4	0	0	0	0
7	35.6	0	0	0	0
8	-1.0	280	17.5	20.6	-5
9	-7.8	0	0	0	0
10	-14.8	0	0	0	0

Note; rates of CH_4 emission from *T. Macrothorax* were 1.072 ng g $termite^{-1}$ s^{-1} and from *C. Fungifaber* were 1.160 ng g $termite^{-1}$ s^{-1}.

8.4.3. Balance between sources and sinks

Does the accumulation of CH_4 from termite point sources, on the landscape scale, exceed the overall sink capacity of the soil? Delmas *et al.* (1992) gave the density of termite mounds within the Mayombe forest (south-western Congo) as 72 ha^{-1} for *T. macrothorax* and 56 ha^{-1} for the unidentified species of *Cubitermes*. If these mound densities are approximately correct for the Mbalmayo Forest Reserve, the termite-related point sources of CH_4 would be approximately 1.9 kg ha^{-1} y^{-1}. Large mounds of other soil feeding termites were also present (eg. those of *Cubitermes heghi*, which produces CH_4 at a rate similar to *T. macrothorax* and *C. fungifaber* (Bignell *et al.*, 1997)); and so total CH_4 release from termite mounds in Cameroon probably exceeds this figure. The mean CH_4 sink strength from the two sampling periods at the old plantation site was small: roughly equivalent to -0.5

Table 8.3 Projections of termite abundance, biomass and the net flux of CH_4 from the mound circle and three concentric surrounding bands. Projections for *C. fungifaber* are for band 1 only.

Location	Termite abundance (workers band^{-1})	Termite biomass (g band^{-1})	CH_4 flux (ng s^{-1})
A. *T. macrothorax*			
In mound	60600	696	636
Under mound (0.13 m^{-2})	3400	39	-
Band 1 (1.64 m^{-2})	2680	30	26
Band 2 (3.14 m^{-2})	609	7	100
Band 3 (4.71 m^{-2})	48	0.6	22
Total	67300	774	784
% outside nest	10	10	14
B. *C. fungifaber*			
In mound	5100	40.9	53
Under mound (0.13 m^{-2})	348	2.8	-
Band 1 (1.64 m^{-2})	1040	9.7	-6
Total	6500	52	48
% outside nest	21	21	-8

kg ha^{-1} y^{-1}. Therefore this site was a net source of about 1.4 kg ha^{-1} y^{-1}. However, there is a large uncertainty associated with the soil sink term, due in part to termite emissions. Moreover, the net flux is likely to be subject to large seasonal fluctuations in the soil sink strength and therefore during the dry season this area may become a net sink. Forest disturbance will also influence the CH_4 budget. Assuming that mound densities are similar in the near-primary and young plantation sites (Eggleton *et al.*, unpublished data), and under the measured seasonal conditions, the young plantation would be a small source of 0.2 kg ha^{-1} y^{-1}, while the near-primary site would be a net sink of -2.8 kg ha^{-1} y^{-1}, clearly showing that the extent to which CH_4 emission from mounds reduces the soil sink strength is dependent on the degree of disturbance to the system.

The net CH_4 flux data collected for the three forested sites plus two measured mounds and transects have been plotted against termite biomass (Figure 8.3). The relationship showed that at high biomass densities, particularly from mounds, termites can contribute significant amounts of CH_4. Across all the sites a termite biomass of 3.8 g m^{-2} was required to turn a source into a sink. This threshold value will vary with soil water content status and level of disturbance.

Termites are important point sources of CH_4 within tropical forests and may influence flux budgets at the landscape scale to an extent dependent on seasonal variations in soil water content and level of forest disturbance.

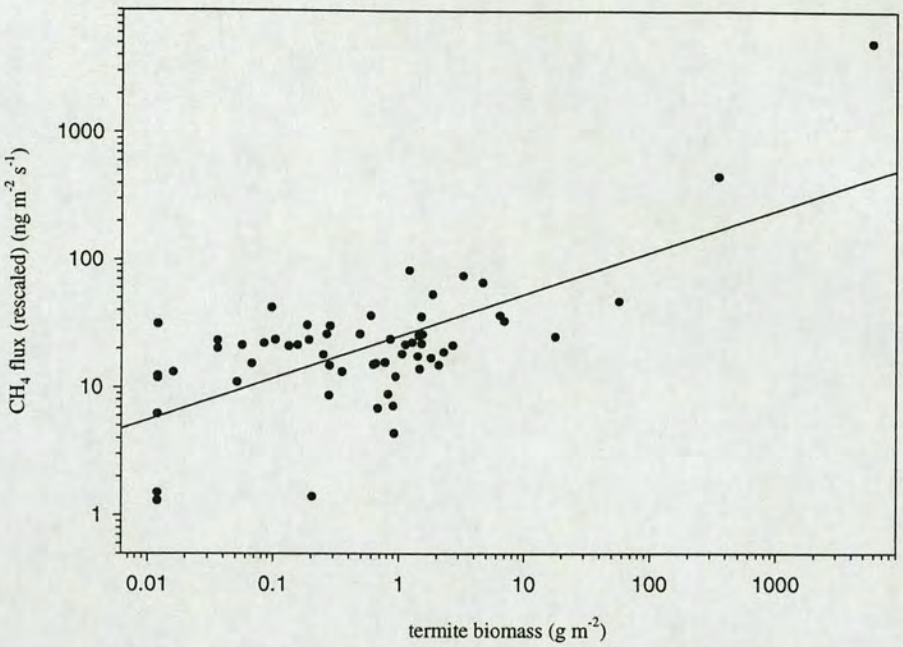

Figure 8.3. The correlation between CH_4 flux and termite biomass for all the forested sites measured including mounds and surrounding area. CH_4 fluxes were rescaled by adding an offset (26) in order to eliminate negative numbers. The regression equation (untransformed) was CH_4 flux (ng m^{-2} s^{-1}) = 0.87× termite biomass (g m^{-2}) - 3.3 ($p<0.001$).

8.5 Summary

Methane flux across a range of forest sites was found to be strongly influenced by disturbance in the form of deforestation and soil feeding termite biomass. Average rates of CH_4 oxidation were approximately 60% smaller from disturbed sites than from a near-primary forest site. This observation was most likely related to the diffusivity of the soil at the latter site, where the soil bulk density was lower and the organic matter content larger than at the disturbed sites. Soil feeding termite biomass was observed to correlate with CH_4 flux, with the direction of flux changing from a net sink to a source for CH_4 at a biomass greater than 3.8 g m^{-2}. Large rates of CH_4 emission up to 636 ng s^{-1} $mound^{-1}$ were observed from termite mounds and at the landscape scale may reduce the soil sink for CH_4 to an extent dependent on the level of disturbance to the forest.

Chapter Nine

The effects of termite biomass and anthropogenic disturbance on the CH_4 budgets of tropical forests in Cameroon and Borneo.

(A version of this chapter has been submitted to the Journal of Tropical Ecology with D. Jeeva, P. Eggleton, R. Davies, D.E. Bignell, D. Fowler, J.H. Lawton and M. Maryati as co-authors)

9.1 Abstract

The exchange of CH_4 between tropical forests and the atmosphere was determined by simultaneously measuring the net CH_4 flux at the soil surface and assessing the flux contribution from soil-feeding termite biomass, both within the soil profile and in mounds.

In Cameroon, both net CH_4 emission and oxidation (-) were observed from the forest soil. CH_4 flux ranged from 40.7 to -53.0 ng m^{-2} s^{-1} in an undisturbed near-primary forest, and from 26.9 to -36.6 ng m^{-2} s^{-1} in an old-growth secondary forest, clear-felled ca 40 years previously. Termite biomass was significantly correlated with CH_4 flux, reversing the direction of flux from a sink for CH_4 to a source at a termite biomass greater than 13.4 g m^{-2} in the secondary forest site, and 18.0 g m^{-2} in the near-primary forest site. Disturbance had a strong effect on the soil sink strength, with the average rate of CH_4 oxidation, at -27.2 ng m^{-2} s^{-1}, being significantly larger at the near-primary forest, than the -17.5 ng m^{-2} s^{-1} observed at the secondary site. Rates of CH_4 emission up to 2000 ng s^{-1} $mound^{-1}$ were measured from termite mounds and termite-derived CH_4 emission reduced the soil sink strength by up to 28 %. CH_4 budgets calculated for each site indicated that both forests were net sinks for CH_4 at -6.1 kg ha^{-1} y^{-1} in the near-primary forest and -3.1 kg ha^{-1} y^{-1} in the secondary forest.

In Borneo, three forest sites representing a disturbance gradient were examined. No net emission of CH_4 to the atmosphere was observed from any of the forest soils. CH_4 oxidation rates were generally lower than those observed in Cameroon and ranged from 0 to -32.1 ng m^{-2} s^{-1}. A significant correlation between the net flux and termite biomass was observed only in an undisturbed primary forest, although the biomass was insufficient to cause net emission of CH_4. Rates of CH_4 oxidation were not significantly different across the disturbance gradient but were, however, larger in the primary forest (averaging -15.4 ng m^{-2} s^{-1}) than in an old-growth secondary forest (-13.9 ng m^{-2} s^{-1}) and a young secondary

re-growth (-10.8 ng m^{-2} s^{-1}). CH_4 flux from termite mounds ranged from net oxidation in an abandoned mound to a maximum emission of 468 ng s^{-1} $mound^{-1}$. CH_4 budgets calculated for each site indicated that CH_4 flux from termite mounds had an insignificant effect on the budget of CH_4 at the regional scale at all three forest sites. Annual oxidation rates were -4.8, -4.2 and -3.4 kg ha^{-1} y^{-1} in the primary, secondary and young secondary forests respectively.

9.2 Introduction

Methane emission by termites and CH_4 oxidation by soil are small but potentially significant components of the global CH_4 budget that have been subject to a wide range of estimates in recent years (Zimmerman *et al.*, 1982, Khalil *et al.*, 1990, Dorr *et al.*, 1993, Potter *et al.*, 1996, Bignell *et al.*, 1997). Investigations into the contribution of termites to the global CH_4 budget have mainly focused on rates of emission from termite mounds (eg. Martius *et al.*, 1993). The most recent estimation of the termite derived CH_4 source strength, by Sanderson (1996), took into account the important differences in CH_4 emission rates between feeding groups of termites (Rouland *et al.*, 1993), resulting in an estimated global source strength of 20 Tg y^{-1}. However, CH_4 emission rates from termites nesting or foraging in the soil were not considered, although soil inhabiting termites represent a potentially large source of CH_4.

The observations made by Eggleton *et al.*, (1996) that the termite population in mounds is a small fraction (<8%) of the overall estimated mean abundance clearly shows the potential for termite-derived CH_4 from forest soil to be a significant CH_4 source. In a previous study in Cameroon MacDonald *et al.*, (1998a) observed a significant correlation between CH_4 flux from the soil and termite biomass across a range of forest sites which had been subject to different levels of disturbance. The ability of termites to affect the soil-atmosphere exchange of CH_4 is dependent on a range of factors which can affect the ability of the soil to oxidise CH_4, namely: soil gas diffusivity, soil water content, and level of habitat disturbance (Dorr *et al.*, 1993, Castro *et al.*, 1995, Keller & Reiners, 1994, MacDonald *et al.*, 1996). MacDonald *et al.*, (1998a) speculated that forest disturbance may have a significant effect on the balance between CH_4 sources and sinks.

During this study a 'whole system' approach was taken in which 50×50 m^2 plots were selected and intensively sampled for CH_4 flux and termite biomass. CH_4 flux measurements were made from a representative selection of surveyed termite mounds. A CH_4 budget was then calculated, for each forest site. As in the MacDonald *et al.*, (1998a) study, field work was again carried out in Cameroon, where the biomass of soil feeding termites is high (Eggleton *et al.*, 1996) and where the influence of termites on the global

CH_4 budget should most easily be seen. A similar study was repeated in Borneo where overall termite biomass was expected to be lower (Eggleton *et al.*, 1994) and where the soil sink strength was unknown.

9.3 Materials and Methods

9.3.1 Site Description

This second investigation in Cameroon was made during March 1996 in the Mbalmayo Forest Reserve (MFR). A description of the reserve, climate and soils is given in MacDonald *et al.*, (1998a) and Chapter 8. Two sites were selected in order to represent undisturbed and disturbed forest and where termite assemblages and biomass were already characterised (Eggleton *et al.*, 1996):

Near-Primary Forest - This plot was subject to selective logging approximately 70 years ago, but retains many large mature trees. Disturbance is very light, confined to local villagers gathering fruits and firewood. A examination of the soil profile at 5 of the chamber sites showed high levels of organic matter, fine roots and macrofauna activity throughout the top 10 cm of the profile. At depths greater than about 15 cm from the surface the soil became predominantly clay.

Secondary Forest - This plot was clearfelled 30 years ago and was left to regenerate, resulting in complete canopy closure. There is more dead wood and woody litter lying on the ground than in the near-primary plot (Eggleton *et al.*, 1996). The soil profile showed significant levels of organic matter and fine roots in the top 5 cm. At depths greater than about 10 cm the soil was predominantly clay.

In April 1996 a similar study was carried out within the Ulu Segama Forest Reserve in SE Sabah, Borneo, Borneo. The reserved comprises the Danum Valley Conservation Area (DVCA), an area of 438 km^2 evergreen, dipterocarp lowland forest including a logged-over area in which approximately 12 stems ha^{-1} have been removed in annual coupes over the last 20 years, thus providing chronosequences of secondary regrowth. Logging is the dominant form of forest disturbance in Sabah (Marsh & Greer, 1992). Soils are classed in the Bang Association (Wright, 1975), the majority being orthic acrisols developed on sandstone and mudstone, with high clay contents. The climate is wet equatorial with an annual rainfall of 2822 mm (5 year average) and a mean annual temperature of 26.7 °C (Marsh & Greer, 1992). Three sites were chosen across a forest disturbance gradient where termite assemblages and biomass had previously been investigated (Eggleton *et al.*, 1997):

Primary forest - This plot was located in an area of undisturbed lowland evergreen dipterocarp forest. Significantly less leaf litter was present on the forest floor than in any of the forest sites in Cameroon. The soil profile showed organic matter and fine roots in a shallow surface layer about 3 cm thick. Below this the soil was predominantly clay.

Old Secondary Forest - This plot was selectively logged in 1978. Regeneration had resulted in an almost complete canopy closure. Understory vegetation was more dense and more leaf litter was present than in the primary forest plot. The soil profile showed organic matter and fine roots in a shallow surface layer similar to the above and about 3 cm thick. Below this the soil was predominantly clay.

Young Secondary Forest - This plot was selectively logged in 1992. The logging resulted in an extremely heterogeneous environment with some undisturbed areas remaining, and others, such as the skid trails where fallen trees are dragged from the forest, resulted in complete destruction of the area and highly compacted soils. The soils in this plot were highly variable, partly because the plot was located on a slope and partly due to the logging disturbance. Generally the soils appeared more organic and to a greater depth, about 5 cm. Canopy cover was incomplete, resulting in some hot and dry areas and there was a large amount of leaf litter and dead wood on the ground.

Primary and disturbed forest sites in coth countries are shown in Plate 9.1.

9.3.2 Chamber measurements

Plots, 50×50 m, were marked out and chamber sites were selected using random co-ordinates. Chambers were inserted into the soil and allowed to equilibrate for periods of at least two hours before the flux measurement was taken. In Cameroon 54 flux measurements were made and in Borneo 20 flux measurements were made at each site. Flux measurements were made using the static chamber method described in Chapter 2. Chambers were enclosed for periods of up to 30 minutes. Samples were stored in 1 l tedlar bags which were air freighted to the UK for analysis of CH_4 by GC within three weeks. Preliminary experiments in which Tedlar bags filled with 2110 ppb CH_4 standard were air-freighted from the UK to Cameroon and Borneo and returned after 3 weeks showed the change in CH_4 concentration was less than 15 ppb. Immediately after the CH_4 measurement had been made soil under each chamber was dug out to a depth of 20 cm and the termites present extracted by hand sorting. Termites were preserved in 70% ethanol for later identification and counting.

9.3.4 Mound measurements

The mounds in each plot were surveyed and CH_4 flux measurements were made on a representative number. The species whose mounds were measured in Cameroon included: *Cubitermes bulbifrons*, *Cubitermes gaigei, Procubitermes arboricola*, *Astalotermes quietus*, *Astalotermes* spp. and *Cephalotermes rectangularis*. In Borneo the species included: *Dicuspiditermes nemorosus, Dicuspiditermes santschii, Bulbitermes* sp. C and *Prohamitermes mirabilis*. Mound fluxes were estimated using direct and indirect methods, depending on the mound size and location. Measurements were made on intact mounds both in the field and the laboratory, using the static chamber technique described above. In Cameroon measurements were also made from sub-sections of mounds and scaled up. For the mounds of *Cubitermes bulbifrons* a significant correlation was observed between mound weight and CH_4 emission rate (r^2=0.984, $p<0.001$), allowing the net flux from these large mounds (which are built against trees) to be estimated. In Borneo the numbers of termites in each mound were also determined either by direct counting or by a sub-sampling procedure (Eggleton *et al.*, 1997) and the CH_4 emission rates were then calculated from the laboratory measurements made from each species of termite identified in the plots.

9.3.5 Isolated termite measurements

Rates of CH_4 emission from individual termites were measured as described in Chapter 8.

9.3.6 Atmospheric CH_4 concentration profiles

Ambient air was sampled through polypropylene tubing at heights above the ground of 0.5, 2 and 5 m. 1 l samples were withdrawn using a gas tight syringe (Hamilton) and stored in tedlar bags. Samples were analysed by TDL spectroscopy. Samples were taken at three intervals between 8 am and 5 pm. Samples were taken at all sites with the exception of the young secondary forest in Borneo.

9.3.7 Soil analysis

Soil temperature at 5 cm depth was routinely measured during flux measurements. Soil water content was determined at each chamber site by % weight loss on drying. Dry bulk density (1-5 and 6-10 cm) was calculated from the mass of oven dry soil in a given volume, five samples were taken from each plot. For Cameroon data for organic carbon, total N and soil pH are from Eggleton *et al.*, (1996). The Borneo pH, organic carbon and total N data are from Homathevi *et al.* (1997).

Plate 9.1. Top - Primary forest, Borneo. Middle - Slashed and burned forest, Cameroon. A mound of *Cubitermes bulbifrons* (~ 3 m high) can be seen in the middle of the picture. Bottom - A static chamber covering a mound of *Dicuspiditermes santschii* in Borneo.

9.4 Results and Discussion

9.4.1 The effect of soil-feeding termite biomass on the CH_4 flux from the forest floor.

In Cameroon, both net CH_4 oxidation and emission were observed from the soil in both forest plots. In the near primary forest CH_4 flux ranged from 40.7 ng m^{-2} s^{-1} to -53.0 ng m^{-2} s^{-1} compared to between 26.9 and -36.6 ng m^{-2} s^{-1} in the secondary forest. CH_4 flux was significantly correlated with termite biomass at both the near primary ($p<0.01$) and secondary sites ($p<0.001$) (Figure 9.1), with the soil sink becoming a source at a termite biomass greater than 18.0 g m^{-2} in the near primary forest and 13.4 g m^{-2} in the old secondary forest. The marked clumping in termite densities (Eggleton *et al.*, 1996) means that intermediate and high biomasses are uncommon and only 4 chambers at each site showed a net emission of CH_4 to the atmosphere. The regression equations describing the relationships in the two forest plots indicated that where no termites were present the soil sink strength would have been -20.8 and -29.2 ng m^{-2} s^{-1} in the secondary and near primary forest plots respectively. Therefore, the termites were mediating the soil sink strength by 3.3 and 2.0 ng m^{-2} s^{-1} or by 16 and 7 %. A similar relationship between CH_4 flux and termite biomass was observed in a previous study (MacDonald *et al.*, 1998a/Chapter 8). However, a biomass of only 3.8 g m^{-2} was required to turn a CH_4 sink into a source, possibly as a result of the measurements being made during the wet season when oxidation rates were at a minimum. Net CH_4 emission from soil has also been observed in several studies in areas of savannah (Scharfe *et al.*, 1990, Poth *et al.*, 1995). Termites have been suggested as the source, although no attempt has yet been made to quantify them.

In Borneo no net emission of CH_4 to the atmosphere was observed from the forest floor and rates of CH_4 oxidation ranged from 0 to -32.1 ng m^{-2} s^{-1} across the three sites. However, a significant correlation was observed between rates of CH_4 oxidation and termite biomass in the primary forest site ($p<0.05$), with high termite biomass reducing the oxidation rate but being insufficient to reverse the direction of flux. Termite biomass was low in all three sites compared to those found in the forests of Cameroon; the majority of chambers contained no termites, and all were below 8 g m^{-2}. Termite generic richness is known to be higher in the Ethiopian biogeographical region than in the Indo-Malayan regions (Eggleton *et al.*, 1994), therefore the above differences in termite biomass between countries was not surprising. The differences in diversity are thought to be due to the degree of climatic disturbance experienced during the Quaternary period (Eggleton *et al.*, 1994). Rates of CH_4 oxidation were not significantly different across the three levels of disturbance ($p>0.05$) but were however, larger in the primary forest, averaging -15.4 ng m^{-2} s^{-1} compared

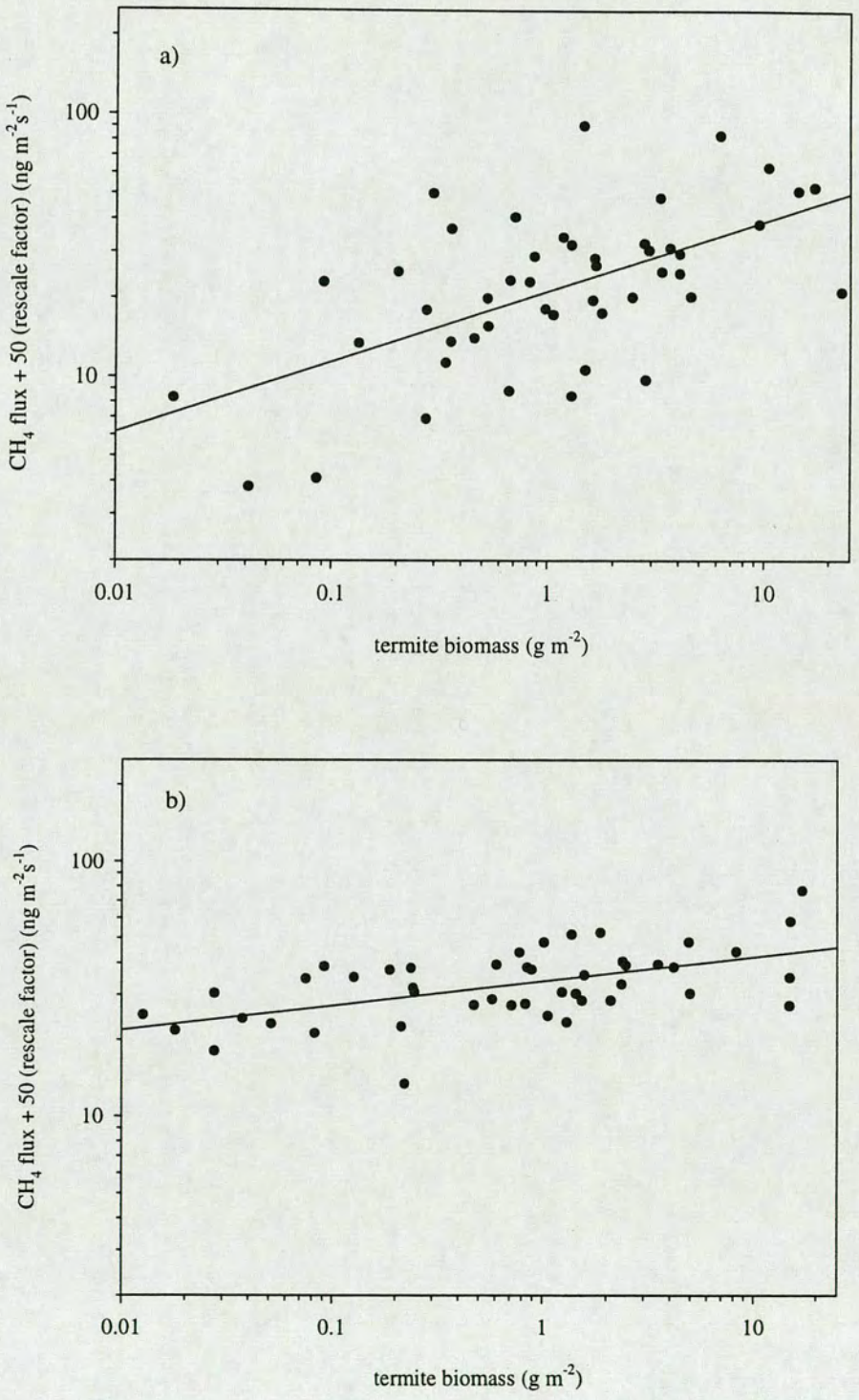

Figure 9.1. The correlation between CH_4 flux and termite biomass in the Cameroon sites a) near primary forest where the regression equation was CH_4 flux (ng m^{-2} s^{-1})= 1.62×termite biomass (g)-29.2 (r^2=0.151, $p<0.01$) and b) secondary forest where the regression equation was CH_4 flux (ng m^{-2} s^{-1})= 156×termite biomass (g)-20.8 (r^2=0.313, $p<0.001$). An offset of 50 was added to all the CH_4 flux data in order to eliminate negative numbers. The regression equations are from the raw untransformed data.

to -13.9 ng m^{-2} s^{-1} in the old secondary forest and -10.8 ng m^{-2} s^{-1} in the young secondary forest.

The net flux of CH_4 between the soil and the atmosphere depends on several factors. CH_4 produced by termites will diffuse along a concentration gradient through the soil, to the atmosphere. The amount of CH_4 oxidised before reaching the atmosphere will depend on the length of the diffusive path, the concentration of CH_4 in the soil, and the ability of the microorganisms in the soil to oxidise the CH_4. CH_4 oxidation rates are known to be concentration dependent (Bender & Conrad, 1995). Despite the increase in termite biomass over several orders of magnitude in Figure 9.1, the corresponding increase in CH_4 emission was relatively small. It seems likely therefore that the rate of CH_4 oxidation in the soil increased as the amount of CH_4 produced by termites increased. Soil conditions affecting gas diffusion through the soil, such as soil texture, organic matter content, bulk density and soil water content (Dorr *et al.,* 1993, Castro *et al.,* 1995, Czeipel *et al.,* 1995, MacDonald *et al.*, 1996) will have an important impact on the net flux. Therefore factors such as disturbance may indirectly affect the amount of termite-derived CH_4 that reaches the atmosphere.

9.5 The effect of disturbance on CH_4 flux from the forest floor.

The mean CH_4 oxidation rate in the secondary forest in Cameroon was significantly ($p<0.05$) smaller at -17.5 ng m^{-2} s^{-1}, than at the near primary site (-27.2 ng m^{-2} s^{-1}), a difference of about 35%. This significant difference was most likely related to disturbance in the form of clear-felling, which occurred 30 years ago. Disturbance in the form of deforestation and conversion to agriculture has been observed to inhibit rates of CH_4 oxidation in several studies, both in tropical and temperate climates (Keller *et al.,* 1990, Keller & Reiners, 1994, Dobbie *et al.,* 1996, Steudler *et al.,* 1996). The exact nature of the inhibition is not well understood but compaction leading to an increase in soil bulk density, and hence decrease in the diffusion of CH_4 to microorganisms has been found to be an important factor (Keller & Reiners, 1994, MacDonald *et al.*, 1996/Chapter 3). Disturbance to the soil profile, altered rates of N cycling, and changes in the microbial population structure may also contribute to the observed inhibition (Mosier *et al.,* 1991, Bender & Conrad, 1994, Priemé *et al.,* 1997). Parameters such as soil water content and temperature were very similar between sites (Table 9.1). Soil bulk density was lower in the secondary than in the near-primary forest, although not significant at $p<0.05$. However, organic carbon was significantly larger ($p<0.05$) in the near-primary than in the secondary forest (Table

9.1). These parameters are indicative of the gas diffusivity of the soil and suggest that the secondary forest had a smaller diffusivity than the near-primary site, possibly as a result of the deforestation. The soil profile observations also suggested a larger diffusivity in the near-primary site, with evidence of fine roots and macrofauna activity to a greater depth than at the secondary site.

In Borneo the largest oxidation rate was observed in the primary forest and the smallest was observed in the most recently disturbed forest plot. However, these differences were not significant. As was observed in Cameroon, the most disturbed site was associated with a high soil bulk density (significant at $p<0.05$) and low organic carbon content (Table 9.1). However, the differences in oxidation rates between undisturbed and disturbed sites were not as pronounced as those observed in Cameroon, probably as a result of logging methods (selective logging versus complete clearance) and/or intrinsic soil properties. The young secondary forest was on a slope and had a slightly lower average soil water content than the other two sites, which could have increased oxidation rates, thereby masking any disturbance effect. A significant correlation between soil water content and CH_4 oxidation rates from each chamber was observed ($r^2 = 0.382$, $p<0.05$) at this site. The logging resulted in a very heterogeneous environment consisting of a range of soil conditions, from relatively undisturbed areas to skid trails where the fallen trees were dragged from the forest resulting in complete destruction of the area and highly compacted soils. Descriptions of each chamber site were noted and scored according to level of disturbance (Table 9.2), and showed that lower rates of CH_4 oxidation (-1.0 ± 1.8 ng m^{-2} s^{-1}) were found in the skid trails and other highly disturbed areas than in the undisturbed areas, where rates of CH_4 oxidation were comparable with those found in the primary forest (-20 ± 8.8 ng m^{-2} s^{-1}).

The degree of inhibition observed between disturbed and undisturbed forests in both countries was fairly low compared with some other studies (Keller *et al.*, 1990, Dobbie *et al.*, 1996). The old secondary sites in both Cameroon and Borneo, had been disturbed 30 and 19 years ago respectively and the forests had been left to regenerate, and this may have resulted in the recovery of the CH_4 oxidising capacity of the soil. Keller & Reiners (1994) observed complete recovery of CH_4 oxidation rates 50 years after deforestation and conversion to pasture in Costa Rica. However, MacDonald *et al.*, (1998a)/Chapter 8 observed no evidence for recovery across a forest disturbance gradient in Cameroon, and in temperate forest Priemé *et al.*, (1997) concluded that recovery of CH_4 oxidation rates to pre-disturbance levels can take over 100 years. The level of disturbance affects not only the net oxidation of CH_4 from the atmosphere by soil but also the ability of the soil to oxidise the

Tabe 9.1. CH_4 flux and soil physical and chemical characteristics from disturbed and undisturbed forest in Cameroon and Borneo.

	CAMEROON - moist pre-montane semi-deciduous forest		BORNEO - wet lowland evergreen dipterocarp forest		
	Near Primary Forest	Secondary Forest	Primary Forest	Old Secondary Forest	Young Secondary Forest
CH_4 flux[1] ($ng\ m^{-2}\ s^{-1}$)	-27.2 (18.6)	-17.5 (11.4)	-15.4 (6.4)	-13.9 (8.4)	-10.8 (9.5)
soil water content (%dry weight)	36.1 (6.3)	33.4 (3.7)	34.4 (5.1)	35.4 (4.9)	31.7 (7.0)
soil temperature (° C)	22.9 (0.4)	23.3 (0.6)	24.8	25.1	25.5
bulk density ($g\ cm^{-3}$)	0.64 (0.12)[2] 0.97 (0.06)	0.72 (0.12) 1.12 (0.17)	0.75 (0.06) 0.98 (0.09)	0.76 (0.07) 1.01 (0.06)	1.0 (0.14) 1.3 (0.12)
pH ($BaCl_2$)	3.5[3] 3.5	3.7 3.8	3.3 -	3.1 -	3.5 -
organic carbon (%)	10.3[3] 3.5	4.5 1.4	3.6 (0.8) -	3.3 (1.3) -	3.0 (2.0) -
total N (%)	0.27[3] 0.20	0.21 0.11	0.33 -	0.25 -	0.22 -

[1]= mean () standard deviation. CH_4 flux measurements = 54 in Cameroon and 20 in Borneo.
[2] = upper figure is for 1-5 cm, lower figure is for 6-10 cm
[3]= upper figure is for top 1-5 cm, lower figure is for 5-50 cm

Table 9.2. Rates of CH_4 oxidation from the young secondary forest in Borneo and the corresponding level of disturbance at each chamber site. The level of disturbance was assessed according to site descriptions at the time of measurement and known history.

Level of disturbance	Mean CH_4 oxidation rate (std. dev.) ($ng\ m^{-2}\ s^{-1}$)	n
1 - highly disturbed	-1.0 (1.8)	4
2 - disturbed	-8.0 (5.3)	9
3 - undisturbed	-20.0 (8.8)	7

1 - the highly disturbed sites included skid trails where there was insignificant canopy cover and the soils were disturbed and/or compacted.
2 - the disturbed sites contained a high proportion of decomposing wood, the vegetation was dense at ground level and the soils were disturbed.
3 - the undisturbed sites were 'secondary forest like' with no large trees but maintaining a good canopy cover with little ground level vegetation and undisturbed soils.

CH_4 produced by the termites within the soil profile. In Cameroon a larger termite biomass was required to turn the forest soil from a net sink to a net source in the near-primary (18.0 $g\ m^{-2}$) than in the old secondary forest (13.4 $g\ m^{-2}$), indicating that disturbance to the soil profile had reduced the capacity of the soil to oxidise the termite CH_4, thereby increasing the contribution of termite-derived CH_4 to the atmosphere.

Disturbance may also affect termite biomass and assemblage composition (Eggleton & Bignell, 1995). A previous study of the two Cameroon sites (Eggleton *et al.*, 1996) showed little difference in overall abundance and biomass, but the secondary succession reduced the proportion of soil-feeding species in favour of wood-feeding forms, which generally produce less CH_4 on a weight-specific basis (Brauman *et al.*, 1992). In Borneo, a similar effect of canopy opening can also be demonstrated (Eggleton *et al.*, 1997). However, the effect on the overall budget is likely to be small.

Overall the rates of CH_4 oxidation observed in Cameroon were at the high end of the range observed in other tropical forest soils, whereas in Borneo the oxidation rates were more comparable with Keller *et al.*, (1990), Tathy *et al.*, (1992) and Keller & Reiners, (1994). A comparison between the CH_4 oxidation rates from all the sites investigated showed that differences between forest sites were as significant as differences between countries and levels of disturbance. Overall, the largest rates of oxidation were observed from the near-primary forest in Cameroon ($p<0.05$), whereas oxidation rates were not significantly different between the secondary forest in Cameroon and the primary or old secondary forests in Borneo ($p>0.05$). The most obvious difference between the forest soils

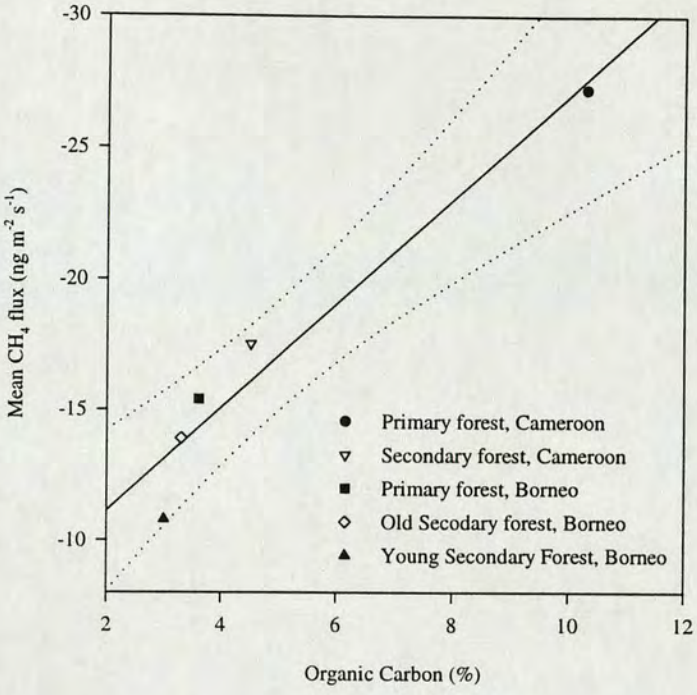

Figure 9.2 The correlation between mean CH_4 oxidation rates at each site and the organic carbon content (1-5 cm) of the soil at each forest site. The regression equation was CH_4 oxidation rate (ng m^{-2} s^{-1}) = -1.98 * organic carbon (%) - 7.16 ($r^2 = 0.944$, $p<0.05$).

studied in the two countries was in their organic carbon content, which was nearly three times larger in the near-primary forest in Cameroon than in the primary forest in Borneo. A significant correlation ($r^2 = 0.944$, $p<0.05$) was observed between the mean CH_4 oxidation rate measured at each site and the organic carbon content of the soil (Figure 9.2). Soil bulk density also varied between the ranged from 0.64 to 1.0 g cm^{-3}. A correlation was observed with the mean oxidation rate from each forest site although it was only marginally significant at $p<0.1$. Another factor which may have affected the intrinsic CH_4 oxidation rate was the termite biomass. The large termite biomass present in the forests of Cameroon, relative to Borneo, may have enhanced rates of CH_4 oxidation by improving soil structure (Brussard & Juma, 1996), and by providing a plentiful supply of substrate for the methane-oxidising community.

9.4.2 Rates of CH_4 emission from termite mounds

Rates of CH_4 emission from the termite mounds spanned a wide range, with rates of up to 2000 ng s^{-1} $mound^{-1}$ being estimated (Tables 9.3 and 9.4). In Cameroon, nearly 3 times as much CH_4 was emitted from the mounds in the secondary forest than from those in the near-

Table 9.3. Rates of CH_4 emission from termite mounds in the a) near primary forest, and the b) secondary forest, Cameroon.

Species	Average mound weight (g)	n (mounds in plot)	Net CH_4 emission (ng s^{-1})
a)			
Astalotermes quietus	66 (49)[1]	22	143
Procubitermes arboricola	484 (424)	10	968
Cephalotermes rectangularis	467	2	467
Cubitermes bulbifrons	10040 (9113)	3	1808
Astalotermes spp.	1048 (405)	8	125
Total		45	**3712**
b)			
Astalotermes quietus	112	19	212
Cubitermes bulbifrons	8940	11	5478
Cubitermes gaigei	9333	3	1660
Procubitermes arboricola	840	1	168
Astalotermes spp.	678	3	81
Cephalotermes rectangularis		1	312
Total		**38**	**9588**

[1]Mean () standard deviation

Note; field measurements were made from each species of termite mound. The number of mounds in the 50×50 m plot were surveyed and the net CH_4 emission rate was estimated by scaling up using mound weights.

9.3). Emission of CH_4 from *Cubitermes bulbifrons* mounds accounted for 90% of the total CH_4 emitted in the secondary forest and 49% in the near primary forest. The rates of CH_4 emission from mounds observed in the present study were comparable to those reported by MacDonald *et al.*, (1998a) for a plantation forest in Cameroon. Generally, the mound building species in the MFR are *Cubitermes bulbifrons*, *Cubitermes fungifaber* and *Thoracotermes macrothorax*. Mound densities and occupation rates (mounds may be abandoned by their constructors and/or colonised by other species of termites) are very variable and incompletely understood (Eggleton & Bignell, 1997). A further complication is that mounds of *Cubitermes bulbifrons* are always built against tree trunks or buttress roots, which makes enclosure and therefore measurement of gas fluxes difficult. Of the other mounds measured (Table 9.3) *Procubitermes arboricola* also uses tree trunks for support, *Astalotermes* mounds are usually small oval structures built around aerial creepers and vines and *Cephalotermes rectangularis* mounds are large structures embedded in the soil. In Borneo CH_4 emission from the mounds was very small compared to the total emission observed from the plots in Cameroon. The total CH_4 emission from mounds was largest from the old secondary forest at 1170 ng s^{-1}, compared to 532 ng s^{-1} in the primary

Table 9.4. Rates of CH_4 emission from the termite mounds found in the **a)** primary forest, **b)** old secondary forest and **c)** young secondary forest 50×50 m plot in Borneo.

a) Species	Mound weight (g)	Termite biomass (g)	CH_4 emission rate (ng s^{-1})	Predicted CH_4 emission rate (ng s^{-1})
Bulbitermes sp C	480	33.6	22.4	38.9
Bulbitermes sp C	778	131	49	151.5
Dicuspiditermes nemorosus	5580	40.4	**7.7**	40.0
Dicuspiditermes nemorosus	5006	55.1	6.9	54.6
Dicuspiditermes santschii	852	18.1	14.4	17.7
Dicuspiditermes santschii	472	4.64	**8.0**	4.53
Dicuspiditermes santschii	264	2.62	4.5	2.57
Total CH_4 emission (ng m^{-2} s^{-1})			**532**	
b) Species				
Prohamitermes mirabilis	1266	1.96	*2.64*	2.64
Prohamitermes mirabilis	1458	0.13	*0.17*	0.17
Dicuspiditermes santschii	1170	48.37	265	47.3
Dicuspiditermes santschii	974	71.13	17.3	69.5
Dicuspiditermes santschii	386	8.12	**50.8**	7.94
Dicuspiditermes santschii	632	10.57	**83.1**	10.3
Dicuspiditermes santschii	3270	14.56	**430**	14.24
Dicuspiditermes nemorosus	1390	75.72	91.7	75.0
Dicuspiditermes nemorosus	1358	17.03	**89.6**	16.9
Dicuspiditermes nemorosus	486	1.74	**32.0**	1.72
Dicuspiditermes nemorosus	960	15.68	**63.3**	15.5
Bulbitermes sp C	214	23.4381	46.7	27.2
Total CH_4 emission (ng m^{-2} s^{-1})			**1172**	
c) Species				
Dicuspiditermes santschii	1880	50.4	157	49.3
Dicuspiditermes santschii	792	7.72	30.9	7.55
Dicuspiditermes santschii	434	1.62	**30.4**	1.58
Dicuspiditermes santschii	224	7.46	**15.7**	7.30
Total CH_4 emission (ng m^{-2} s^{-1})			**234**	

normal typescript indicates that values were measured in the field.
bold typescript indicates that values were scaled up using the weights of mounds from measured values.
italic typescript indicates that values were scaled up from termite CH_4 emission rates measured in the lab.

forest and only 234 ng s^{-1} in the young secondary forest (Table 9.5). The CH_4 emission rates measured directly using the static chamber technique were generally larger (in 8 out of 11 cases) than those predicted from the measurements of termite species in the laboratory, possibly as a result of the termites producing more CH_4 when in contact with mound material as suggested by Bignell *et al.*, (1997).

9.4.3 Vertical profiles of CH_4 in the atmosphere

Vertical profile concentration measurements have previously shown significant gradients of CH_4 in the air over flooded and dry tropical forest, indicating the net source strength of the surrounding area (Delmas *et al.*, 1992, Tathy *et al.*,1992). The temperature inversion between the ground and the lower part of the canopy which occurs during days of high insolation induces high air stability in the lower part of the forest (Delmas *et al.*, 1992, Tathy *et al.*, 1992). After sunset, when temperature gradients diminish, ventilation will occur. Vertical profiles of CH_4 from each site are shown in Fig. 9.5. The gradients observed here were small and very variable. At the near-primary forest site three out of the six gradients measured suggested a net soil sink for CH_4 with difference in concentration of up to 20 ppb between 0.5 m and 2.0 m above the ground. The 8.30 am gradient on the 14 March had a CH_4 concentration 22 ppb lower at 5 m than at 2 m and 0.5m, possibly indicating the concentration after ventilation to the free atmosphere during the night. Generally CH_4 concentrations increased throughout the day within and outwith the forest. During the early morning sampling, CH_4 concentrations were similar within and outwith the forest, by midday concentrations of CH_4 were larger in than out of the forest, but by 5 pm concentrations outside the forest were larger than inside the forest by up to 24 ppb. These results suggest that termites may be influencing CH_4 concentrations on the landscape scale, but there is a more significant source outwith the forest, which, by the end of the day has influenced the CH_4 concentrations within the forest. This source is unknown, the seasonally flooded forest was very dry at this time of year, and cattle are not grazed locally, due to tsetse flies. Emissions of CH_4 from permanent wetlands is a possibility. At the secondary site the profiles were not so well defined, and the weather conditions were not as suitable, therefore only 3 gradients over 2 days were measured. No significant gradient between 0.5 m and 2.0 m was observed, which may be a result of the diminished soil sink due to deforestation. Concentrations were similar within and outwith the forest, except on the morning of the first sampling where the concentration outwith the forest was 22 ppb greater than the concentration at ground level, again suggesting a CH_4 source outwith the forest. In Borneo CH_4 concentrations were very similar at each height indicating the low CH_4 source and sink strengths.

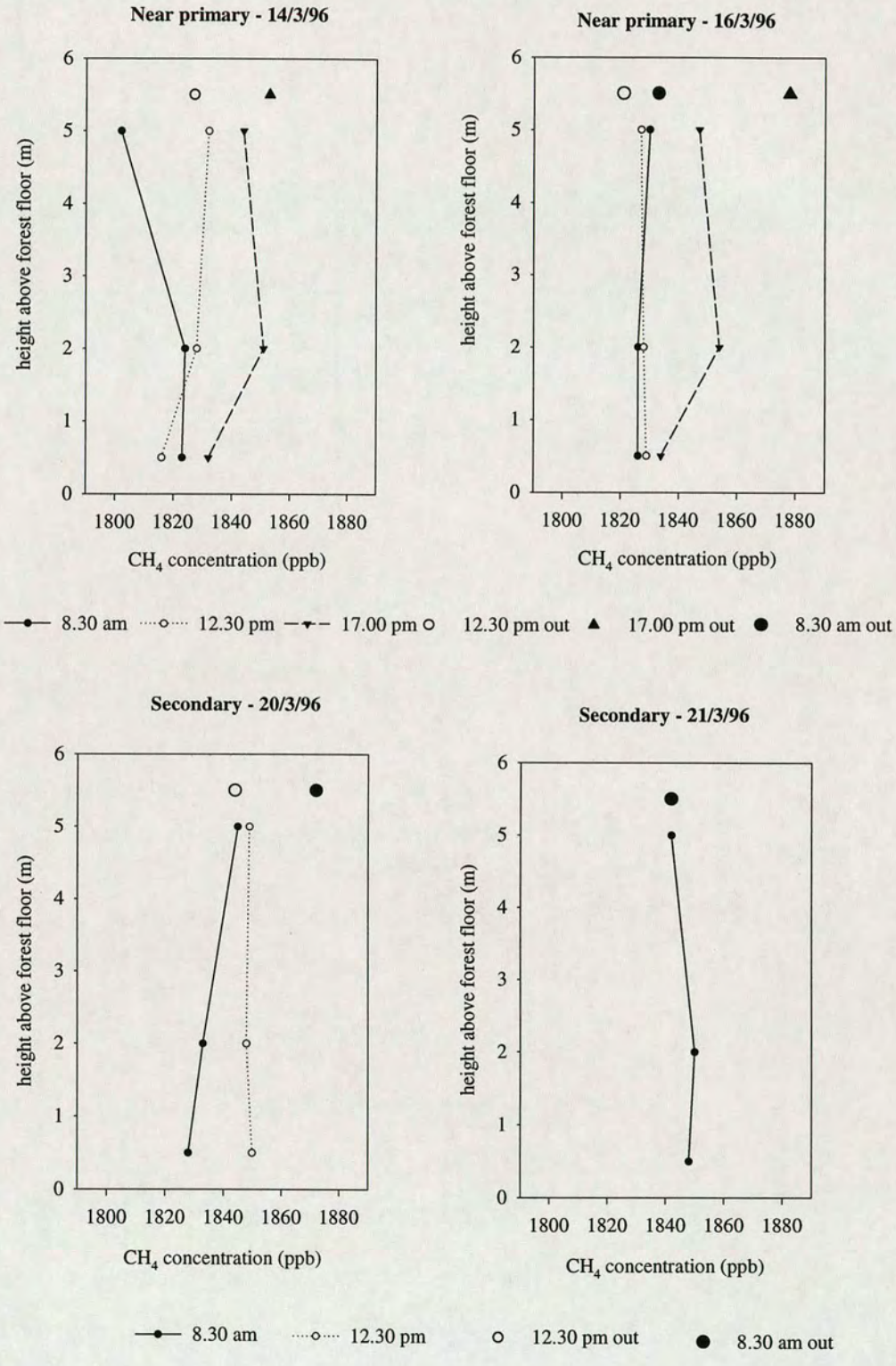

Figure 9.5 Vertical profiles of atmospheric CH_4 concentrations within disturbed and undisturbed forest in Cameroon

Table 9.5. The CH_4 budget from a) near primary and secondary forest in Cameroon, and b) primary, old secondary and young secondary forest in Borneo.

	Mean CH_4 oxidation rate (kg ha^{-1} y^{-1})	Total CH_4 emission from mounds (kg ha^{-1} y^{-1})	Net CH_4 flux (kg ha^{-1} y^{-1})
a)			
Near Primary Forest	-6.6 (-8.6)*	0.5	**-6.1** (-8.11)
Secondary Forest	-4.3 (-5.5)	1.2	**-3.1** (-4.3)
b)			
Primary Forest	-4.9	0.1	**-4.8**
Old Secondary Forest	-4.4	0.2	**-4.2**
Young Secondary Forest	-3.4	0.0	**-3.4**

*values in brackets represent the net flux prior to the seasonal adjustment.

9.4.4 The CH_4 budget at each site

All the forest plots investigated were net sinks for CH_4 (Table 9.5). However, termite biomass in both mounds and soil, and disturbance (deforestation) reduced the soil sink strength to varying degrees summarised in Table 9.6. The seasonal variation in rates of CH_4 oxidation in Cameroon is likely to be large. In MacDonald *et al.*, (1998a) measurements made at the end of the rainy season showed rates of CH_4 oxidation at the near-primary site over 50% smaller than those observed during the present study, at the end of the dry season. In order to estimate annual oxidation rates, the mean flux observed during this study was averaged with the mean flux observed in MacDonald *et al.*, (1998a)/Chapter 8 for the near primary forest. For the secondary site the same seasonal variability (54 %), was assumed. The seasonal adjustment resulted in a net soil sink of -6.6 and -4.3 kg ha^{-1} y^{-1} in the near-primary and secondary sites respectively. The CH_4 emission from termite mounds was assumed to be constant and with the seasonal variation in precipitation, and a consequent adjustment to the estimates of CH_4 oxidation rates, the net flux was -6.1 kg ha^{-1} y^{-1} in the near primary forest and -3.1 kg ha^{-1} y^{-1} in the secondary forest (Table 9.5). CH_4 emission from termite mounds had the most significant effect on the net flux in the secondary forest where the CH_4 emitted by all the soil feeding termite mounds in the plot reduced the soil sink by 28 % (an amount similar to the observed inhibition as a result of the deforestation).

Table 9.6. The effects of CH_4 production by termites (mounds and soil) and disturbance (clearfelling and selective logging) on the soil sink strength expressed as a percentage relative to the mean CH_4 oxidation rate.

Process	%
termite CH_4 production in soil	0 - 16
termite CH_4 emission from mounds	1 - 28
forest disturbance	10 - 35

Termite biomass in the soil profile reduced the net soil sink by between 7 and 16%. In Borneo, termite biomass did not have a significant effect on the soil sink strength. The CH_4 emitted through the termite mounds reduced the CH_4 oxidation rate by only 0.1 - 4 % (Table 9.5). In Borneo the climate is wet equatorial and the seasonality is weak, therefore strong variations in CH_4 uptake rate are unlikely. However, it should be noted that during the field measurements the climate was exceptionally dry. Nevertheless, no adjustment for seasonal variation in CH_4 oxidation rates was made. The primary forest had an annual oxidation rate of -4.8 kg ha^{-1}, compared to -4.4 kg ha^{-1} at the old secondary site and -3.4 kg ha^{-1} in the young secondary forest. In a recent estimate of the global CH_4 sink strength Potter *et al.,* (1996), calculated, from a model computation and from extrapolation of measured fluxes respectively, that tropical seasonal forest oxidised CH_4 at rates of -1.7 and -2.3 kg ha^{-1} y^{-1}. The results from the Cameroon forests suggest these fluxes may be a significant underestimation, with an annual CH_4 sink in the undisturbed forest of -6.6 kg ha^{-1} y^{-1}, and -4.3 kg ha^{-1} y^{-1} in the disturbed sites. The oxidation rates observed in Borneo were in good agreement with the values reported by Potter et al., (1996) for tropical rainforests, at between -3.4 and -4.9 kg $ha^{-1}y^{-1}$.

9.5 Summary

The net CH_4 flux was estimated from semi-deciduous and wet tropical forests in Cameroon and Borneo respectively. Measurements of the soil-atmosphere exchange were made from undisturbed primary forest and from forest sites which had experienced different levels of disturbance. In Cameroon both net CH_4 emission and oxidation were observed with fluxes ranging from 40.7 to -53.0 ng m^{-2} s^{-1}. The CH_4 flux was found to be significantly correlated with termite biomass which reduced the soil sink strength by up to 16%. In Borneo the net flux to the forest floor ranged from 0 to -32.1 ng m^{-2} s^{-1}, no net emission of CH_4 was observed. Termite biomass had no effect on the net flux. Disturbance in the form of

deforestation was observed to inhibit rates of CH_4 oxidation by between 10 and 35%, principally as a result of changes in the gas diffusivity status of the soil. Termite mounds produced CH_4 at a rate of up to 2000 ng s^{-1} $mound^{-1}$ and reduced the soil sink by up to 28% in Cameroon and 4% in Borneo. All the forest sites were net sinks for CH_4 of between -3.05 and -6.15 kg ha^{-1} y^{-1}.

Chapter Ten

Discussion

10.1 The range of CH_4 flux in the environments studied.

Methane flux from the sites studied covered a wide range, from a net oxidation of -53.0 ng m^{-2} s^{-1} in undisturbed semi-deciduous rainforest in Cameroon, to a net emission of 780 ng m^{-2} s^{-1} through the vascular plant *Menyanthes trifoliata* in pool areas of blanket bog in Caithness, Scotland. The CH_4 fluxes from sites studied are shown, together with representative values from the literature, in Table 10.1. In general, the rates of net CH_4 oxidation observed in this study were in close agreement with the values observed in the literature, which range from 0 to about -70.0 ng m^{-2} s^{-1} (for references see Table 10.1). This range is small when compared with CH_4 fluxes from wetlands which can range from -9.2 to 11200 ng m^{-2} s^{-1} (Bartlett & Harriss, 1993). A schematic representation of the CH_4 fluxes measured from the range environments studied is shown in Fig. 10.1.

Temperate forest soils are one of the most extensively studied environments for the oxidation of CH_4. Rates of CH_4 oxidation observed in the temperate forest soils in this study (-0.1 to -14.6 ng m^{-2} s^{-1}) were at the low end of the range of values observed in other studies eg. Crill, (1991), Dobbie *et al.,* (1996). This may have been a consequence of inhibition of CH_4 oxidation rates due to disturbance, as all the forests investigated in this study were plantations or shelterbelts on ex-agricultural land. However, there are comparable values for temperate forests in the literature (Castro *et al.,* 1993, Lessard *et al.,* 1994). In a recent review of global CH_4 oxidation rates Potter *et al.,* (1996) estimated that cool temperate forests oxidised on average -4.1 and -14.4 ng m^{-2} s^{-1} (calculated from a global model and an extrapolation of measured fluxes respectively), in good agreement with the range of values observed in this study.

In contrast to temperate forests, rates of CH_4 oxidation from agricultural land were generally small and covered a narrow range, from 0.5 to -5.9 ng m^{-2} s^{-1}, in good agreement with the literature (Table 10.1). Net CH_4 emission to the atmosphere was periodically observed from some agricultural sites although the rates were small, generally less than 0.5 ng m^{-2} s^{-1}. Low rates of CH_4 oxidation from agricultural land relative to forests have been observed from a range of soil types and land uses (Lessard *et al.,* 1994, Flessa *et al.,* 1995).

Table 10.1 Measurements of CH_4 flux in the field using static chambers.

Ecosystem	Location	Average CH_4 flux ($ng\ m^{-2}\ s^{-1}$)	Range ($ng\ m^{-2}\ s^{-1}$)	Reference
temperate forest	North America	-19.1	2.3 - -56.7	Crill 1991
temperate forest	Germany	-	0- -68.9	Dorr *et al.*, 1993
temperate forest	North America	-42.5	-3.0 - -63.9	Castro *et al.*, 1995
temperate forest	UK	-6.2	-0.1 - -14.6	this study[1]
temperate forest	Canada	-5.8	-0.5 - -12.7	Lessard *et al.*, 1994
arable		-0.3	0 - -1.5	
temperate forest	UK	-16.2	-2.2 - -38.2	Dobbie *et al.*, 1996
arable		-8.1	-0.1 - -21.7	
arable	Germany	-1.8	0 - -6.3	Flessa *et al.*, 1995
arable land	UK	-0.9	0.5 - -5.9	this study[2]
grassland	North America	-8.0	-5.5 - -9.7	Mosier *et al.*, 1991
grassland	UK	-8.7	-2.5 - -18.8	this study[3]
grazed grassland		-1.8	0.8 - -4.4	
seasonal tropical forest	Panama	-	-4.0 - -9.0	Keller *et al.*, 1990
pasture	Panama	-	-1.6 - -2.3	
seasonal tropical forest	Cameroon	-22.1	40.7 - -53.0	this study[4]
disturbed forest		-11.9	26.9 - -36.6	

tropical wet forest	Costa Rica	-14.6	-	Keller & Reiners, 1994
secondary forest		-13.9	-	
pasture		2.4	-	
tropical moist forest	Brazil	-19.8	-	Steudler et al, 1996
pasture		11.4	-	
tropical wet forest	Borneo	-15.4	-4.4 - -24.9	this study[5]
secondary forest		-12.3	0 - -32.1	
tundra	North America	-2.7	1162.5 - -20.8	Whalen *et al.*, 1991
moorland	UK	0.5	35.9 - -16.7	this study[6]
wetland	UK	173.3	18.2 - 780.4	this study[7]
wetland	Canada	238	-1.1 - 1620	Roulet *et al.*, 1992
wetland	North America	2303	208 - 10023	Crill *et al.*, 1988

[1] average of annual oxidation rate at Dunslair and average oxidation rate at Glencorse, Devilla, North Berwick and Springfield Farm forested sites.
[2] average of Springfield Farm barley field, North Berwick oil seed rape and East Linton winter wheat.
[3] grassland at Glencorse, grazed grassland at Springfield Farm, Fell gate grass, carex and Sunnit grass at Great Dun Fell (mean of summer and winter values).
[4] average of wet and dry seasons flux, disturbed sites are average of all the disturbed sites measured at Mbalmayo during the wet and dry seasons.
[5] secondary forest flux is the average of the young and old secondary forest.
[6] average flux from moorland at Dunslair and moorland at Loch More.
[7] mean value was measured by micrometeorological techniques and is from Hargreaves & Fowler (1997). The range is representative of the environments measured in Caithness and at the Mine Road at Great Dun Fell (mean of winter and summer values).

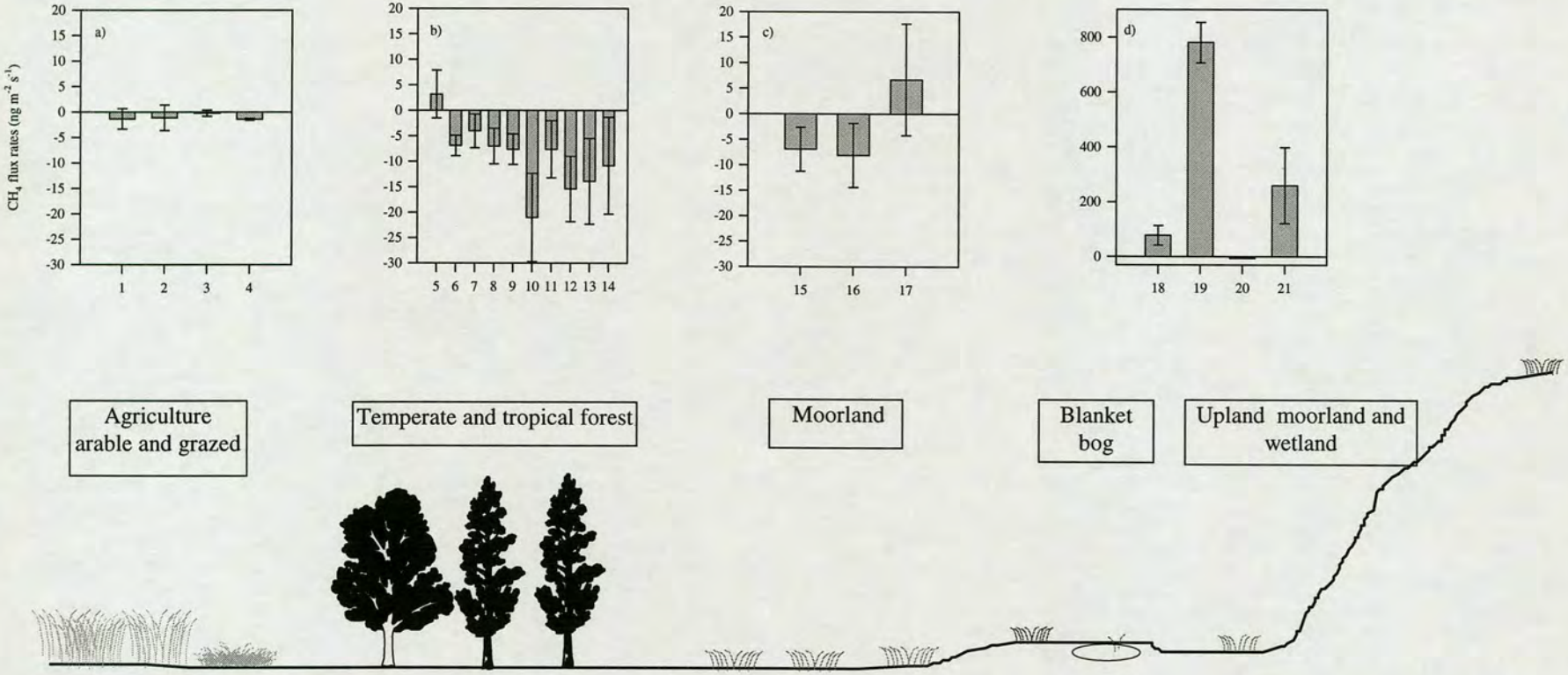

Figure 10.1 Schematic representation of the CH_4 flux range from the environments studied. a) CH_4 flux from agricultural land: 1 grazed grass and 2 barley field at Springfield Farm, 3 oil seed rape near North Berwick (June) and 4 winter wheat near East Linton (April/May) b) CH_4 flux from temperate and tropical forest: 5 plantation forest in Caithness, 6 shelter belt at North Berwick (June), 7 shelter belt at Springfield Farm, 8 Devilla, 9 Glencorse, 10 Primary forest, Cameroon, 11 Secondary forest, Cameroon, 12 Primary forest, Borneo, 13 Secondary forest, Borneo, 14 Young secondary forest, Borneo. c) CH_4 flux from moorland: 15 Dunslair 1994, 16 Dunslair, 1995, 17 bog heather moor, Caithness. d) CH_4 flux from upland moorland and wetland: 18 minimum (pool edge) and 19 maximum (pool vascular transport) values from Caithness blanket bog, 20 minimum (Fell Gate *juncus*) and 21 maximum (summit *carex* pool).

Dobbie *et al.,* (1996) reported oxidation rates from an agricultural soil in Scotland averaging -14.7 ng m^{-2} s^{-1}, significantly larger than those for this study, probably as a result of the soil texture. However, compared to an adjacent forest soil these rates were relatively small. Grasslands showed similar rates of oxidation, with the ex-agricultural grassland in Scotland comparing well with a grassland in North America (Mosier *et al.,* 1992). The grazed grassland showed much smaller CH_4 oxidation rates, comparable with the values observed from the arable land (Table 10.1). In this study the average reduction in oxidation rates from agricultural land relative to forests was over 80%, at the high end of the range observed by Dobbie *et al.*, (1997) (from a range of European soils) who calculated that the conversion of forest to agriculture has led to a decrease in the soil sink strength of 2.3 Tg y^{-1}.

Rates of CH_4 oxidation from tropical forests covered a wider range of CH_4 flux values than those observed in temperate forests (Table 10.1). The largest range in CH_4 flux was observed in Cameroon, where net emission rates of up to 40.7 ng m^{-2} s^{-1} and oxidation rates of up to -53.0 ng m^{-2} s^{-1} were measured. The oxidation of CH_4 was found to be significantly reduced by emissions of CH_4 by termites and deforestation. Investigations into CH_4 fluxes in the tropics and subtropics are few relative to those in temperate climates. In a recent estimate of the global sink strength, Potter *et al.,* (1996) calculated, from a model and from extrapolation of measured fluxes respectively, that tropical seasonal forest oxidised CH_4 at a rate of 1.7 and 2.3 kg ha^{-1} y^{-1}. The results from the Cameroon study suggest this flux may be a significant underestimate, with an annual CH_4 sink in the undisturbed forest of 6.6 kg ha^{-1} y^{-1}, and 4.3 kg ha^{-1} y^{-1} in the disturbed sites. Flux measurements from wet tropical forests covered a smaller range (0 to -32.1 ng m^{-2} s^{-1}) and the values reported by Keller & Reiners (1994) and Steudler *et al.,* (1996) agree well with the oxidation rates observed from the rainforest in Borneo, which had an annual sink strength between 3.4 and 4.8 kg ha^{-1} y^{-1}. Using the above annual uptake rates and forest cover data for the moist deciduous and rainforest zones (FAO, 1993), the contribution of these areas and the effect of land use change on the global sink strength was calculated (Table 10.2). The annual uptake rates for Cameroon (which include seasonal variability) were assumed to be representative of the moist deciduous zone to give a pre-deforestation soil sink strength of 8.6 Tg y^{-1}. The present day soil sink (which included estimates of CH_4 oxidation rate from disturbed areas) was estimated at 6.6 Tg y^{-1}, a reduction of about 25%. There are, of course, many problems associated with this type of extrapolation, not least the scarcity of data from these regions. Rates of CH_4 oxidation are also concentration-dependent and therefore pre-disturbance oxidation rates may not have been as large as those observed today for a particular soil.

Table 10.2 Forest cover, deforestation rates and past and present CH_4 sink strengths for the moist deciduous forest and rainforest zones.

Forest Zone	Land Area (million ha)	Forest cover 1990 (million ha)	Annual deforestation rate 1981-1990 (%)	Original CH_4 sink strength ($Tg\ y^{-1}$)	Present CH_4 sink strength ($Tg\ y^{-1}$)
Moist Deciduous	1298.6	587.3	1.0	8.9	6.6
Rainforest	937.1	718.3	0.6	4.5	4.2

Data for land area, forest cover and deforestation rates from FAO 1993.
Value for the present soil sink includes estimated oxidation rate from disturbed land.

Another complicating factor is the rate of oxidation from disturbed areas, which is dependent on the time since disturbance. Uptake rates in the rainforest zone extrapolated from the Borneo data resulted in a sink strength of 4.2 $Tg\ y^{-1}$ and the disturbance had only a small effect, a reduction of about 7%. The rainforest zone data agree well with values in the literature and the global extrapolation of Potter et al., (1996). However, more significant effects of disturbance, and even a reversal in the direction of flux, have been observed in other studies (Keler & Reiners, 1994, Steudler *et al.*, 1996). The total CH_4 uptake rate in the moist deciduous and rainforest zones for 1990 was estimated at 10.8 $Tg\ y^{-1}$, or 36% of the total soil sink for CH_4 (as estimated by IPCC, 1995).

The largest range in CH_4 flux was observed from tundra, moorland and wetland sites (Table 10.1). Whalen *et al.,* (1991) observed CH_4 consumption to be the dominant process from tundra in North America, with sporadic point sources providing sources of CH_4. In this study the two moorland sites showed very different CH_4 fluxes, as a result of differences in soil water content, with net oxidation being observed at Dunslair, which had a soil water content of about 135 % (dry weight), and net emission at Loch More, with a soil water content of about 560% (dry weight). Rates of CH_4 emission from wetlands covered a wide range from 18 to 780 $ng\ m^{-2}\ s^{-1}$ at the sites studied. Comparable wide ranges have been found in most other studies (see Table 10.1 for examples).

Methane fluxes for the ecosystems discussed above (with the exception of the wetlands) covered a fairly similar range. Methane oxidation rates were as variable within sites as between similar and contrasting land use types (Table 10.1). For example, the range observed by Dorr *et al.,* (1993) from differently textured forest soils in Germany was of a similar magnitude to the difference in oxidation rates observed between adjacent forested and cultivated land (Dobbie *et al.,* 1996, Lessard *et al.,* 1994). No clear differences were

apparent between climate zones, with similar oxidation rates in tropical and temperate forests, suggesting that in the majority of aerobic mineral soils, soil temperature does not control the rate of CH_4 oxidation.

10.2 Processes controlling spatial variability in CH_4 flux

In the preceding chapters it was shown that soil water content, bulk density, N input, temperature and land use had a significant effect on rates of CH_4 oxidation in a range of soils. The importance of each environmental factor on CH_4 oxidation rates varied between sites. For example, at Dunslair (Chapter 4) soil temperature controlled seasonal changes in oxidation rates while N input had a strong effect on spatial variability. At North Berwick (Chapter 3) land use strongly affected CH_4 oxidation, with rates on average 80% smaller from an oil seed rape field than from the soil in a shelterbelt. In peat, water content and temperature were observed to have important effects on the magnitude and net direction of CH_4 flux.

In order to investigate the processes controlling spatial variability in oxidation rates between and within sites, mean values from each site were correlated with all available soil physical and chemical characteristics. Sites were split into mineral and peat soils, in view of the very different soil properties such as soil water content and bulk density between the two soil types. For the mineral soils, annually averaged values from each site were used where possible, with the exception of the East Linton arable, and North Berwick oilseed rape and shelter belt sites, where annual rates were not available. For the tropical sites, mean values for wet and dry seasons were used where possible.

10.2.2 Gaseous diffusion

Soil bulk density, was significantly (negatively) correlated with rates of CH_4 oxidation ($p<0.001$) (Figure 10.2), and provides strong evidence that the gaseous diffusion of CH_4 to methanotrophs was the primary controlling factor effecting oxidation rates in these soils. Soil bulk density was found to be correlated with CH_4 oxidation rates in preceding chapters; however, it is remarkable that the correlation above holds for such a broad range of soils and locations including sites in Cameroon and Borneo as well as the UK. Also included in Figure 10.2 are data for disturbed and undisturbed forest in Central America (Keller & Reiners, 1994, Reiners *et al.,* 1994) and in forest and cultivated land in Canada (Lessard *et al.,* 1994) (annual and yearly averaged values respectively) and show very good agreement with the values observed in this study (Figure10.2). Soil bulk density may therefore be used

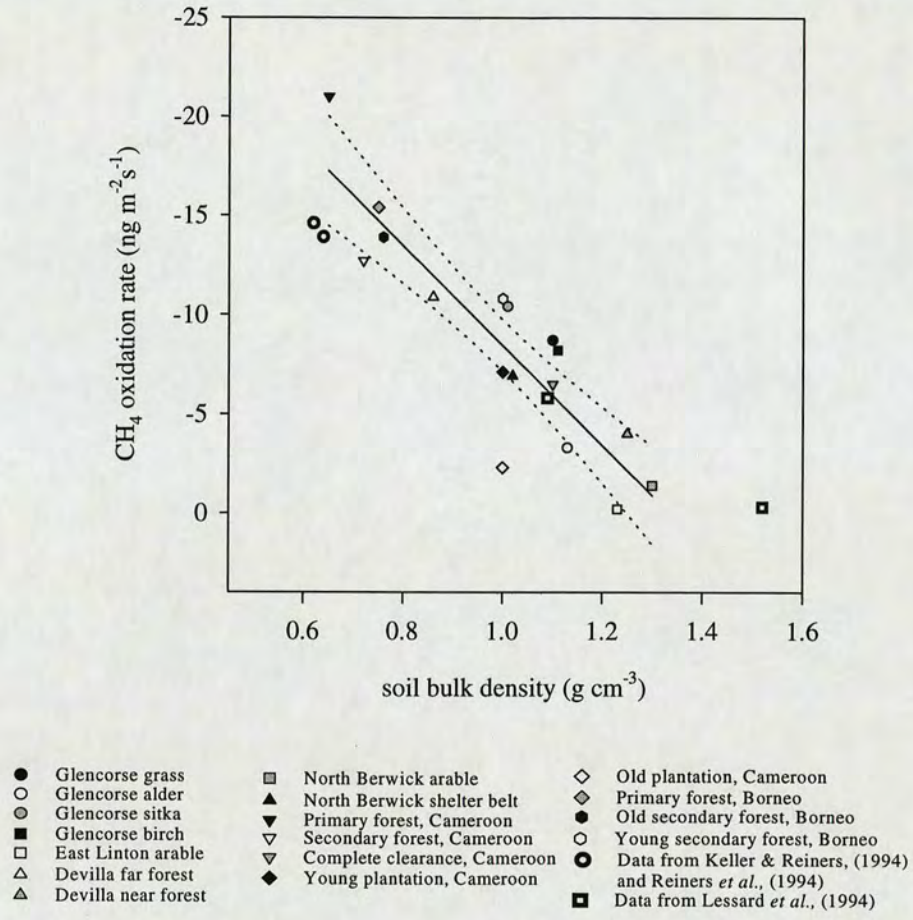

Figure 10.2 The regression between CH_4 oxidation rates and soil bulk density from forest, agricultural and grassland mineral soils in the UK, Cameroon and Borneo. Data have also been added but not included in the regression from Costa Rica and Canada from separate studies (see references above). The regression equation is: CH_4 oxidation rate (ng m^{-2} s^{-1}) = 25.1×bulk density (g cm^{-3})-33.6 (r^2 = 0.786 $p<0.001$). The dotted lines represent the 95 % confidence interval.

as a predictor of CH_4 oxidation rates in mineral soils. Generally, the lowest rates of oxidation were found in agricultural soils which had the largest soil bulk densities, indicating that compaction caused by cultivation was significantly limiting rates of oxidation. The largest rates of CH_4 oxidation were observed at the undisturbed tropical forest sites. The low soil bulk densities observed at these sites in the top 5 cm of soil, reflected the organic matter, macrofauna activity, termite activity and fine roots concentrated in the surface layers of soil.

Table 10.3 The effects of N inputs on soil available NO_3-N, NH_4-N and inhibition of CH_4 oxidation rates. Values are expressed as % reduction relative to control sites.

Site	Mean CH_4 oxidation rate from control sites (ng m^{-2} s^{-1})	N input (kg ha N y^{-1})	Soil available NO_3-N (:g g dry $soil^{-1}$)	Soil available NH_4-N (:g g dry $soil^{-1}$)	inhibition (%)
Glencorse grass	-9.3	265	32.0	37.1	**23**
Glencorse alder	-8.2	150 - 300[a]	6.0	3.1	**0 - 60**[b]
Dunslair forest 615 m	-4.1/-5.7	46.3	1.7	17.0	**46 - 61**[c]
Dunlair soil columns;					
NH_4NO_3	-20.1	40	16.9	14.9	**87**
$NaNO_3$	-20.1	40	27.1	6.2	**86**
NH_4Cl	-20.1	40	1.6	23.9	**70**
NaCl	-20.1	40	1.4	5.3	**75**
Devilla forest	-7.9	5.7	1.5	5.6	**0**

[a] data from Stettler (1978)
[b] range shows the possible extent of the inhibition caused by the N fixing alder sp. (relative to the adjacent birch plot) and therefore the extent of the inhibition is not certain (Chapter 5).
[c] the inhibition observed at the high altitude forest was 46% and 61%, relative to the low altitude forest and moorland respectively.

10.2.3 Soil nitrogen

One of the main factors influencing CH_4 oxidation rates was N input. The extent of the inhibition varied between soils. For example, application of 265 kg N ha^{-1} inhibited CH_4 oxidation rates at the grass site at Glencorse by about 23%, whereas application of only 40 kg N ha^{-1} inhibited rates of CH_4 oxidation by up to 80% in the peat at Dunslair. The results are summarised in Table 10.3. It is apparent that neither N input or soil available NO_3-N and NH_4-N concentrations dominated the variability in the magnitude of the inhibition. The main difference was in the extent of the inhibition between the peat soils at Dunslair (inhibition 46 - 86%) and the mineral soils at Glencorse and Devilla (inhibition < 60%). This may be a result of either a greater sensitivity to N input in the peat soils or, as previously discussed in Chapter 5, that the mineral soils had been previously disturbed by agriculture which resulted in a long-term inhibition of oxidation rates and therefore any further disturbance or N inputs had only a small effect. The mechanism responsible for the inhibition is not well understood, there are conflicting reports on the nature, magnitude, persitence and factors responsible for the inhibition in the literature (Adamsen & King, 1993, Dunfield & Knowles. 1995, Sitaula *et al.,* 1995). The absence of any correlation between soil available

NH_4-N and the observed inhibition at any of the relevant sites in this study suggests that competitive inhibition between NH_4 oxidisers and CH_4 oxidisers was not an important, or at least, not the only mechanism causing the inhibition. Furthermore, the results from the N addition experiment on the soil columns from Dunslair suggested that N itself was not solely responsible for the inhibition and other compounds such as NaCl can inhibit CH_4 oxidation rates to a comparable extent. The mechanism may be due to a toxicity effect (by the production of NO_2^-, for example) or sensitivity to osmotic potential, as discussed in Chapter 4.

Another consequence of acid deposition is the possible effect of S deposition. Inputs of S were observed to inhibit CH_4 emission rates from peat monoliths by up to 50% as a result of competition between methanogens and sulphate-reducing bacteria, discussed in Chapter 7. The extent of the effect of pollutant deposition on CH_4 flux on a global scale is unknown. With total N deposition rates of up to 37.8 kg ha^{-1} y^{-1} and oxidised S deposition rates of up to 92.8 kg ha^{-1} y^{-1} in central Europe (EMEP, 1996), the potential for perturbation to CH_4 fluxes is high. The magnitude of the deposition effect will primarily depend upon the ecosystem and land use type; for example, the deposition of N to agricultural land where CH_4 oxidation rates are already inhibited will have only a small effect compared to the effect in; for instance, forest soils. Also, the relative effects of the different ions, eg. SO_4^{2-} and NH_4^+, on the various microbial grops within a wetland will have important consequences on the net flux. Deposition rates and ecosystem types/land uses need to be mapped in order to estimate the size of the inhibition effect.

10.2.3 Land use change

Disturbance, as a consequence of deforestation and/or agricultural practices, was also observed to decrease CH_4 oxidation rates from a wide range of sites in both tropical and temperate climates. The extent of the inhibition ranged from 10 to 90% (Table 10.4). The only consistent difference between the disturbed and undisturbed sites was soil bulk density, which was higher in all the disturbed than in the undisturbed sites. The correlation between bulk density and CH_4 oxidation rates (Figure 10.2) suggests that the majority of the observed inhibition was a result of a change in the gaseous diffusion in the soils and that compaction limited the diffusion of CH_4 to methanotrophs. The largest bulk densities and CH_4 oxidation rates were found in the agricultural soils; however, after ploughing, these soils will have relatively low soil bulk densities. It seems unlikely that CH_4 oxidation rates will increase in response to this lowering of bulk density, indicating that other factors, such as changes in

Table 10.4 The inhibition of CH_4 oxidation rates as a result of land use (agriculture and deforestation). Values are expressed as % reduction relative to undisturbed sites.

Site	Mean CH_4 oxidation rates from undisturbed sites (ng m^{-2} s^{-1})	inhibition (%)
Springfield Farm - barley field	-4.0	**72**
- grazed grassland	-4.0	**67**
North Berwick - oilseed rape	-6.9	**80**
Cameroon - weeded chromaleana fallow	-14.2	**69**
young plantation forest	-14.2	**66**
old plantation forest	-14.2	**89**
old secondary forest	-27.2	**36**
Borneo - young secondary forest[a]	-15.4	**29**
old secondary forest[a]	-15.4	**10**

[a]not significant at 95%

the microbial population structure, may also contribute to the observed small fluxes. There is evidence to suggest that following disturbance CH_4 oxidation rates take a long time to recover to their pre-disturbance levels (Priemé *et al.,* 1997) and King & Schnell (1994) have suggested that the persistence of the inhibition is related to the inability of methanotrophs to grow or recover on ordinary ambient concentrations of atmospheric CH_4. Therefore it is likely that after the initial disturbance and inhibition of oxidation rates further soil structural changes will have little or no effect. The input of fertiliser to the agricultural soils would also have contributed to the inhibition, although the results shown in Table 10.3 suggest this contribution was smaller than the effects of compaction. Other studies of the effect of deforestation and/or conversion to agriculture have observed inhibitions of a similar magnitude to those observed here (Keller *et al.,* 1993, Dobbie *et al.,* 1996) and Hansen *et al.,* (1993) observed a 50% inhibition due to either compaction or fertilisation, and a 78% inhibition when the two treatments were combined in an agricultural soil.

The peat and organic soils (Dunslair, Loch More, Springfield Farm, Great Dun Fell) showed a much larger range of CH_4 exchange with the atmosphere than the mineral soils, with significant rates of CH_4 oxidation up to -16.6 ng m^{-2} s^{-1} being observed at Dunslair to the largest flux of 780 ng m^{-2} s^{-1} observed in the blanket bogs of Caithness. Fluxes also covered a large range within the same site, for example at Great Dun Fell (Chapter 7). In order to examine the factors determining spatial variability, CH_4 fluxes from all the peat and

organic soils were correlated with the environmental variables measured. Fluxes were selected at a similar time of year, April - June, in order to avoid seasonal variability. Soil water content controlled rates of CH_4 flux within several sites; however, no significant correlations were observed between CH_4 flux and the any of the environmental parameters measured. The relationship observed in Chapter 3 between CH_4 emission rates and soil water content did not hold when the data from Great Dun Fell or from the 'wet' Caithness sites were added. Soil water content has been shown to be the dominant factor affecting rates of CH_4 emission in several studies (Dise *et al.,* 1993, Roulet *et al.,* 1993). However, in this study other factors such as peat depth, substrate supply and oxidising capacity of the surface peat layers must have strongly affected the CH_4 flux.

10.3 Processes controlling temporal variability in CH_4 flux.

Rates of CH_4 oxidation exhibited seasonal variability at all the sites where measurements were made over a time period long enough to include changes in water content and temperature (Dunslair, Glencorse, Devilla and Springfield Farm). Figure 10.3 shows the temperature response of CH_4 oxidation rates from all the sites where statistically significant temperature responses were observed (Glencorse, Devilla (near NH_3), Springfield Farm (grazed grass, shelter belt). An exponential curve gave the best fit through the data points ($r^2 = 0.362$, $p<0.001$). The activation energy was 85.4 kJ mol^{-1} and the Q_{10} value was 3.6. This value was larger than those generally observed for CH_4 oxidation rates (Crill, 1991, Lessard *et al.,* 1994) as a result of the strong temperature dependence observed at Dunslair (Chapter 4). The mineral soils on their own showed a weaker response to temperature, with an activation energy of 59.3 kJ mol^{-1} and a Q_{10} value of 2.4. However, CH_4 oxidation rates were also strongly correlated with soil water content in the mineral soils ($r^2 = 0.568$, $p<0.001$) therefore this response was not due solely to changing temperature. The response of CH_4 oxidation rates to soil water content is shown in Figure 10.4 for the sites where significant responses were observed (Glencorse, Devilla (far NH_3), Springfield Farm (barley field and grazed grassland)) ($r^2=0.568$, $p<0.001$). This linear response which was averaged over seven sites masks the non-linear response observed for example, at the grassland at Glencorse, where as discussed previously (Chapter 5) soil type and diffusivity were important in determining the non-linearity. Both linear and non-linear responses to soil water content have been observed in several studies (Lessard *et al.,* 1994, Czeipel *et al.,* 1995, Dunfield *et al.,* 1995).

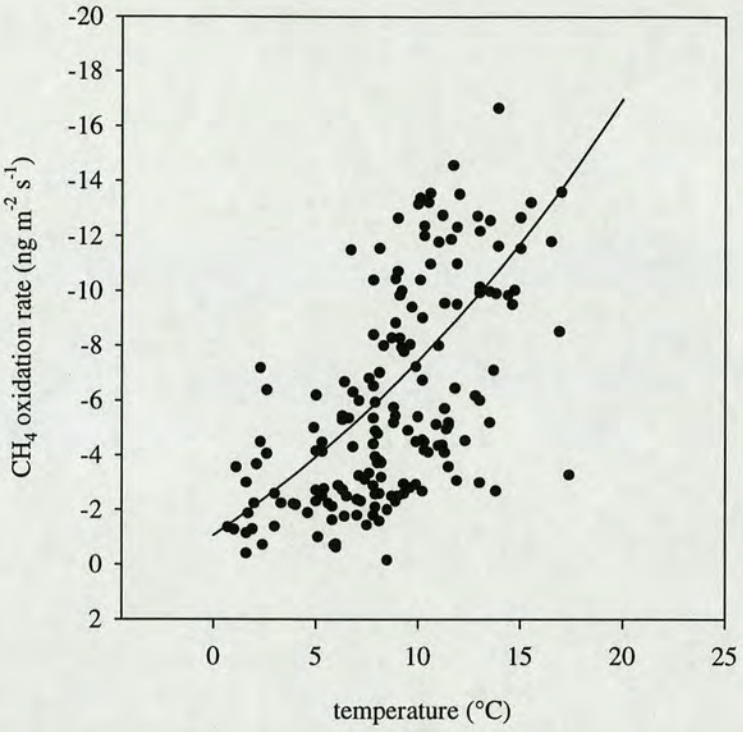

Figure 10.2 The temperature response of CH_4 oxidation rates at Glencorse, Devilla forest, Springfield Farm shelter belt and Dunslair Heights. The regression equation describing the relationship is: CH_4 oxidation rate (ng m^{-2} s^{-1}) = -13.0exp(0.04*soil temperature (°C))+11.9. (r^2 = 0.362, $p<0.001$)

It is clear that both soil water content and soil temperature can significantly influence the CH_4 flux on a temporal basis. The balance between the relative effects of temperature and water content varied with time of year and type of soil, with the dominant control apparently being effected by soil type and diffusivity. In general, soil water content has beeen observed to exert the dominant control on seasonal changes in oxidation rates, with soil temperature showing only a weak effect, attributed to the strong diffusional control on CH_4 oxidation rates (King & Adamsen, 1992, Dorr *et al.*, 1993, Lessard *et al.*, 1994). However, at Dunslair the seasonal change in CH_4 oxidation rates was determined by temperature. Laboratory experiments confirmed that soil water content, at the levels observed in the field, had no effect on CH_4 oxidation rates. This was attributed to the high diffusivity of the peat which allowed a plentiful supply of substrate throughout the year, allowing the methanotrophs to respond to changes in temperature. In the mineral soils at Glencorse soil water content appeared to have the dominant effect on CH_4 oxidation rates, by restricting the diffusion of CH_4 to methanotrophs. However, soil temperature showed a stronger effect in spring. Crill (1991) also observed a strong temperature response in the

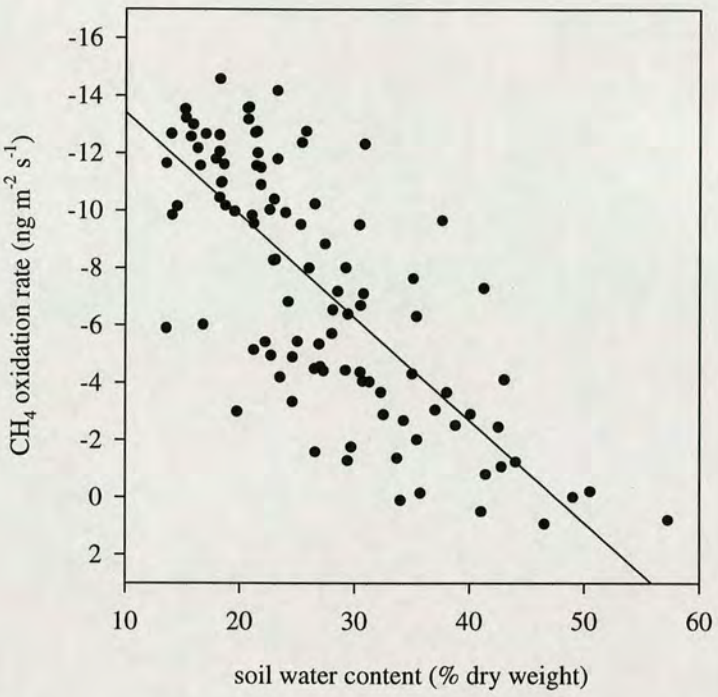

Figure 10.3 The response of CH_4 oxidation rates to seasonal variations in soil water content at Glencorse, Devilla forest (far NH_4) and Springfield Farm (barley field and grazed grassland). The regression equation describing the relationship is: CH_4 flux (ng m^{-2} s^{-1}) = 0.4*soil water content (% dry weight)-17.0 (r^2 = 0.568, $p<0.001$).

spring and suggested that a change from biological to diffusional control was responsible for this observation.

Detailed information on the response of methanotrophs in different soils to changes in soil water content and temperature can be used to estimate the likely effects of climate change on the soil sink for CH_4. For example, changes in temperature will have a much larger effect on the magnitude of the soil sink in soils such as peaty podzols and organic soils than in mineral soils, providing the soil water content does not increase above the level where diffusion becomes the limiting factor. In contrast, mineral soils would be more significantly influenced by soil water content because they are more diffusion limited. Wetlands are likely to be strongly affected by changes in climate Although CH_4 emission rates are principally governed by water contents, soil temperature also has a strong effect. If water regimes remain constant a small increase in temperature of 3 °C could increase the estimated contribution from Northern wetlands fron 34 Tg y^{-1} (Bartlett & Harriss, 1993) to 54 Tg y^{-1}. The major controls affecting spatial and temporal variation in CH_4 flux are summarised in Figure 10.4.

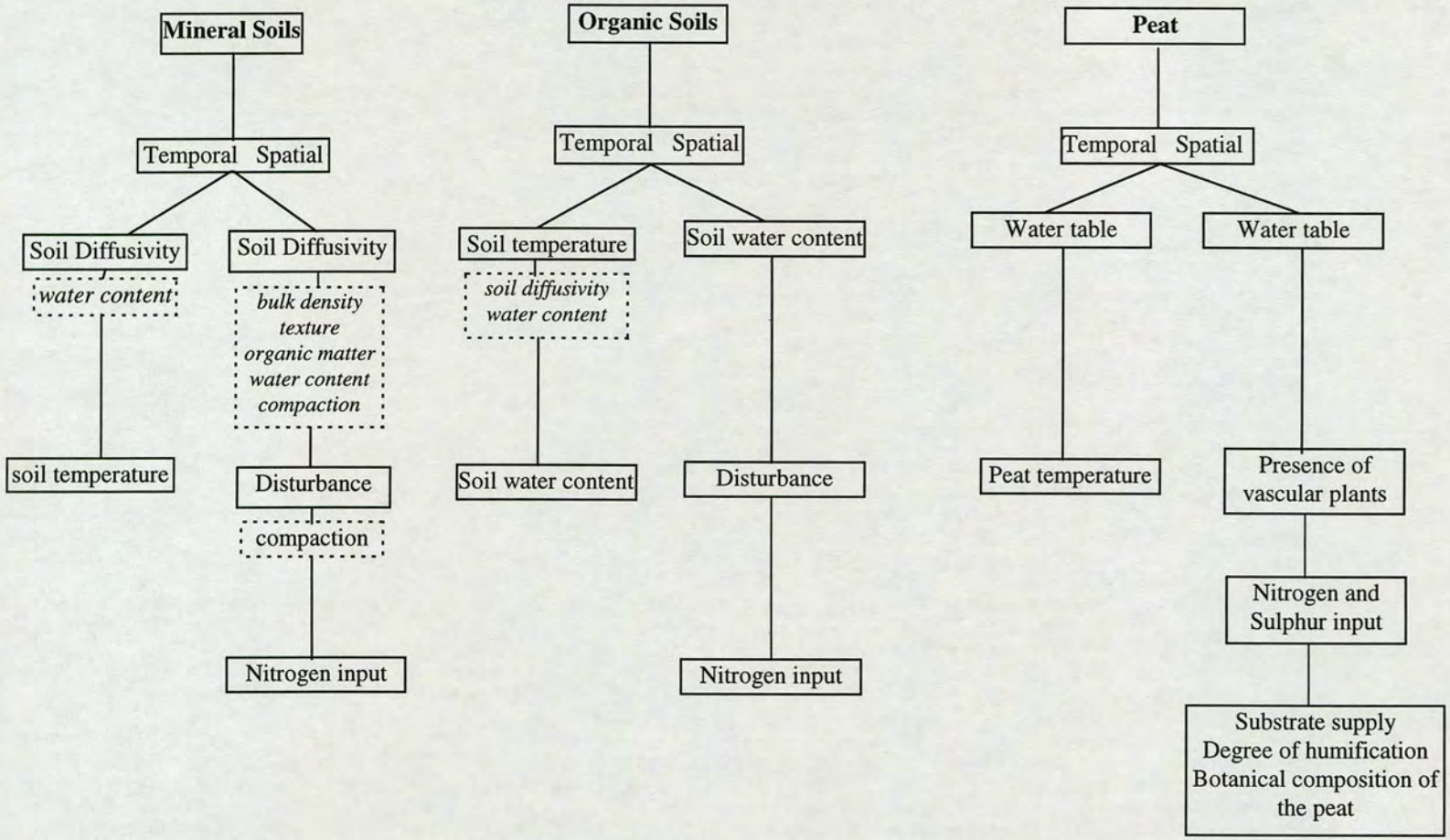

Figure 10.4 Schematic representaion of the the enviromental factors which can effect CH_4 flux from mineral, organic and peat soils. Factors are listed in descending order of importance. Soil characteristics in italics indicate the variables that the controlling factors are dependent on. Note: spatial variability assumes constant temperature.

10.4 Priorities for future work

- Methane flux measurements in the tropics are poorly represented in the literature. Seasonally dry and savannah ecosystems have been identified by Potter *et al.,* (1996) as being under-represented and in an estimate of global emissions from wetlands Bartlett & Harriss (1993) referenced only seven studies from tropical wetlands.

- The effect of soil nitrogen (and other compounds) on the underlying mechanism of CH_4 oxidation rates is in need of further investigation to understand the processes. Other questions such as: is the inhibition dose related? and do rates of CH_4 oxidation recover? have yet to be answered.

- The effect of land use on CH_4 oxidation rates is well established. However, the mechanisms controlling the inhibition are unclear and in need of further investigation. For example, in the case of conversion to agriculture the effect of elevated N input relative to compaction should be investigated along with subsequent effects on the microbial community. The factors responsible for the recovery time of oxidation rates to pre-disturbance levels must be investigated.

- The effect of deforestation on CH_4 oxidation rates in tropical forests needs to be further investigated and the consequences for the global CH_4 budget calculated. In particular the recovery time of CH_4 oxidation rates and the factors influencing recovery time should be examined.

- The role of methanotrophs in mediating CH_4 emission from wetlands, termites and landfills and the effects of disturbance on this sink have not been adequately investigated. The potential for increasing the CH_4 source strength due to the inhibition of CH_4 oxidation from environments such as wetlands is large.

- Seasonal variability of CH_4 flux from wetlands needs to be monitored in order to accurately predict annual emission rates. Seasonal variation in the vascular transport of CH_4 may be of particular importance on an annual basis.

- The mode of release and stomatal control of CH_4 emission through a wide range of wetland plants should be investigated.

- The often significant discrepancy between CH_4 emission rates from termite mounds scaled up from laboratory measurements and those measured in the field should be examined.

- The effect of N and S deposition can mediate the CH_4 flux from wetlands. Investiations into the effects of these elements on the microbal comminity and on net fluxes need to be carried out at realistic application rates. In particular the relative effects on the CH_4 oxidising and producing communities should be investigated.

- The effects of the inhibitory compounds identified in this study need to quantified on a global scale in order to assess the importance of pollutant deposition on CH_4 emission and oxidation rates and to quantify this effect in terms of the global CH_4 budget.

- Mechanistic models of the CH_4 sink strength should take into account the importance of seasonal variation, soil bulk density, the impacts of land use change, N deposition, net CH_4 emission from some agricultural soils and net CH_4 emission from some tropical forest soils due to termites.

Chapter eleven

Summary and conclusions

- The net surface-atmosphere exchange of CH_4 was measured from a wide variety of ecosystems in temperate and tropical climates. In aerobic soils CH_4 was oxidised at rates of up to -27 ng m^{-2} s^{-1} and values were in close agreement with those in the literature (Table 10.1). Sporadic emissions of up to 40 ng m^{-2} s^{-1} were also observed from some aerobic soils and were related to agricultural practices, soil water content and termite biomass. The CH_4 flux from peat soils showed both net oxidation and emission ranging from -16.6 to 780 ng m^{-2} s^{-1} with the magnitude and direction of the flux primarily being dependent on the water content of the peat.

- For mineral soils, spatial variability in CH_4 oxidation rates was closely associated with variability in soil bulk density, reflecting the control of gaseous diffusion of CH_4 to methane oxidisers exerted by the soil porosity. A highly significant negative correlation was observed between soil bulk density and CH_4 oxidation rates in the UK, Cameroon and Borneo such that CH_4 flux (ng m^{-2} s^{-1}) = 25.1 * bulk density (g cm^{-3}) - 35.6 (r^2 = 0.786, $p<0.001$). Values in the literature from Canada (Lessard *et al.,* 1994) and Costa Rica (Keller & Reiners, 1994) showed good agreement with the relationship observed in this study. Soil bulk density may therefore be used as a predictor of CH_4 oxidation rates.

- For peat, soil water content controlled the within-site magnitude and direction of CH_4 flux. However, no single environmental variable was correlated with CH_4 flux across all the sites studied, reflecting the importance of other parameters which influence CH_4 fluxes, including peat depth, subtrate quality and vascular transport.

- Temporal variation in surface-atmosphere CH_4 fluxes was influenced by seasonal changes in soil water content and/or soil temperature. In organic soils, which showed net CH_4 oxidation, soil temperature exerted the primary control and soil water content did not significantly affect oxidation rates at the water contents commonly observed in the field. This was an important observation and was attributed to a high soil gas diffusivity

in these relatively dry peaty soils. In contrast, temporal variation of CH_4 oxidation rates in 'wet' peat was controlled mainly by soil water content, which determined the magnitude and direction of flux. In mineral soils both soil water content and soil temperature affected the flux, although it appeared that soil water content exerted the dominant control. These findings have important consequences for quantifying the effects of climate change on CH_4 fluxes.

- The largest measured CH_4 emission rates (780.0 ng m^{-2} s^{-1}) were emitted through the vascular plant *Menyanthes trifoliata* in pool areas of blanket bog in Caithness, Scotland. Studies under controlled conditions in the laboratory showed that the majority of CH_4 was emitted through the stem and stomata did not play a significant role in controlling the flux.

- Investigations into the effect of temperature on rates of CH_4 emission from peat monoliths showed large positive responses. The emission rates of CH_4 increased exponentially with increasing temperature in the range 5 to 30 °C. The average activation energy and Q_{10} value was 63.5 kJ mol^{-1} and 2.6 respectively.

- Investigations into the effect of sulphate deposition (100 kg S ha^{-1} y^{-1}) on peat showed that rates of CH_4 emission were inhibited by up to 50%, and Na_2SO_4 had a larger effect than $(NH_4)_2SO_4$.

- Inputs of N to soils which were net oxidisers of CH_4 significantly inhibited rates of uptake. Both fertilisation and atmospheric deposition inhibited rates of CH_4 oxidation by between 20 and 60% in the field and inhibitions of up to 80% were observed in a laboratory investigation. The commonly cited hypothesis that CH_4 and NH_4^+ are in competition was not consistent with the results of this study, in which additions of the salts commonly applied with the N compounds resulted in a similar inhibition and no relationship was observed between soil NH_4^+ concentrations and the extent of the inhibition. It is more likely that changes in N turnover resulting in NO_2^- production, or sensitivity of microorganisms to changes in osmotic potential, may be inhibiting methanotrophs or that another as yet unknown mechanism is exerting a toxic effect.

- Land use change was shown to significantly decrease CH_4 oxidation rates. The smallest rates of CH_4 oxidation were consistently observed from agricultural sites. Measurements from shelter belts adjacent to oilseed rape, barley and grazed grass fields indicated that the conversion of forest to agriculture had resulted in a reduction in the sink capacity of the soil between 65 and 80%.

- Land use also strongly affected rates of CH_4 oxidation in tropical forests. Rates of CH_4 oxidation in disturbed sites (plantation forest, complete clearance, commercial logging) relative to primary forest sites were inhibited by between 10 and 90%. It was calculated that deforestation in the moist deciduous zone may have reduced the soil sink for CH_4 by 25%.

- The total CH_4 uptake in the moist deciduous and rainforest zones was estimated at 10.8 Tg y^{-1} or 36% of the global terrestrial soil sink for CH_4 (as estimated by IPCC, 1995).

- The small bulk densities observed in all the disturbed, relative to the undisturbed, sites suggests that compaction, resulting in a restriction of the diffusion of CH_4 to methane oxidisers, was the main factor responsible for the observed inhibition of CH_4 oxidation rates. However, other factors such as fertilisation, changes in nutrient dynamics and changes in microbial population structure may also have contributed to the described effects.

- Methane production by termites influenced the net CH_4 flux from the forest floor at several sites in Cameroon. Significant positive correlations were observed between termite biomass and CH_4 flux, with the direction of flux changing from a net sink to a net source at a termite biomass between 3.4 and 18 g m^{-2}, depending on soil water content and level of disturbance. The termite-derived CH_4 reduced the soil sink strength by up to 16 %. In Borneo termite biomass was small and no significant effect on the soil sink strength was observed.

- Rates of CH_4 emission of up to 2000 ng s^{-1} $mound^{-1}$ (252 ng m^{-2} s^{-1}) from various termite mounds were observed. At the landscape scale it was calculated that CH_4 emission from termite mounds could mediate the soil sink strength by up to 28 % in Cameroon, in Borneo the emission was insignificant.

- The oxidation of CH_4 by soil is a small but important component of the global CH_4 budget. It is inhibited by many land-use related anthropogenic activities and has the potential to respond to predicted changes in climate.

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